(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 December 2002 (12.12.2002)

PCT

(10) International Publication Number
WO 02/099040 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number: PCT/US02/17313

(22) International Filing Date: 3 June 2002 (03.06.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/296,076	5 June 2001 (05.06.2001)	US
60/328,605	10 October 2001 (10.10.2001)	US
60/338,733	22 October 2001 (22.10.2001)	US
60/357,253	15 February 2002 (15.02.2002)	US
60/357,600	15 February 2002 (15.02.2002)	US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/099040 A2

(54) Title: IGS AS MODIFIERS OF THE P53 PATHWAY AND METHODS OF USE

(57) Abstract: Human IG genes are identified as modulators of the p53 pathway, and thus are therapeutic targets for disorders associated with defective p53 function. Methods for identifying modulators of p53, comprising screening for agents that modulate the activity of IG are provided.

IGs AS MODIFIERS OF THE p53 PATHWAY AND METHODS OF USE

REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. provisional patent applications 60/296,076 filed 6/5/2001, 60/328,605 filed 10/10/2001, 60/338,733 filed 10/22/2001, 60/357,253 filed 2/15/2002, and 60/357,600 filed 2/15/2002. The contents of the prior applications are hereby incorporated in their entirety.

BACKGROUND OF THE INVENTION

The p53 gene is mutated in over 50 different types of human cancers, including familial and spontaneous cancers, and is believed to be the most commonly mutated gene in human cancer (Zambetti and Levine, FASEB (1993) 7:855-865; Hollstein, *et al.*, Nucleic Acids Res. (1994) 22:3551-3555). Greater than 90% of mutations in the p53 gene are missense mutations that alter a single amino acid that inactivates p53 function. Aberrant forms of human p53 are associated with poor prognosis, more aggressive tumors, metastasis, and short survival rates (Mitsudomi et al., Clin Cancer Res 2000 Oct; 6(10):4055-63; Koshland, Science (1993) 262:1953).

The human p53 protein normally functions as a central integrator of signals including DNA damage, hypoxia, nucleotide deprivation, and oncogene activation (Prives, Cell (1998) 95:5-8). In response to these signals, p53 protein levels are greatly increased with the result that the accumulated p53 activates cell cycle arrest or apoptosis depending on the nature and strength of these signals. Indeed, multiple lines of experimental evidence have pointed to a key role for p53 as a tumor suppressor (Levine, Cell (1997) 88:323-331). For example, homozygous p53 "knockout" mice are developmentally normal but exhibit nearly 100% incidence of neoplasia in the first year of life (Donehower *et al.*, Nature (1992) 356:215-221).

The biochemical mechanisms and pathways through which p53 functions in normal and cancerous cells are not fully understood, but one clearly important aspect of p53 function is its activity as a gene-specific transcriptional activator. Among the genes with known p53-response elements are several with well-characterized roles in either regulation of the cell cycle or apoptosis, including GADD45, p21/Waf1/Cip1, cyclin G, Bax, IGF-BP3, and MDM2 (Levine, Cell (1997) 88:323-331).

The cell-cell adhesion system at cadherin-based cell-cell adherens junctions (AJs) consists of at least one nectin and an l-afadin. Nectin is a Ca(2+)-independent homophilic

immunoglobulin-like adhesion molecule, and l-afadin is an actin filament-binding protein connecting the cytoplasmic region of nectin to the actin cytoskeleton (Tachibana, K. et al. (2000) J Cell Biol; 150(5): 1161-76). The trans-interaction of both nectin and the interaction of nectin with l-afadin are required for their colocalization with E-cadherin and catenins at Ajs (Tachibana, K. et al. (2000) *supra*). Nectin and cadherin interact through their cytoplasmic domain-associated proteins and possibly these two cell-cell adhesion systems cooperatively organize cell-cell Ajs (Tachibana, K. et al. (2000) *supra*). Nectins are also part of the immunoglobulin superfamily, are homologues of the poliovirus receptor, and are also named poliovirus receptor-related (PRR) proteins (Reymond, N.et al. (2001) J Biol Chem; 276(46): 43205-15). The poliovirus receptor (PVR) is an integral membrane glycoprotein, which plays an important role in allowing the poliovirus to enter a cell. Its extracellular region contains 3 immunoglobulin-like domains. Two integral forms, PVR-alpha and PVR-delta, and 2 soluble forms, PVR-beta and PVR-gamma, lack a transmembrane domain generated by alternative splicing of mRNA. The normal cellular function of PVR is unclear (Eberle, F. et al. (1995) Gene 159: 267-272).

Poliovirus receptor-related 1 (PVRL1 or Nectin1) is an immunoglobulin-related cell adhesion molecule, which mediates cellular entry for many alpha herpes viruses (Reymond, N.et al. (2001) *supra*). Autosomal recessive mutation in the corresponding gene is linked to cleft lip/palate-ectodermal dysplasia (Tachibana, K. et al. (2000) *supra*).

Poliovirus receptor-related 2 (PVRL2 or Nectin2) is a transmembrane glycoprotein and member of the nectin family that shows cell-cell adhesion activity (Eberle, F. et al. (1995) *supra*). It may function as a coreceptor for mutant herpes simplex virus types 1 and 2 and pseudorabies virus (Reymond, N.et al. (2001) *supra*). The PVRL2 gene encodes 2 glycoproteins, PVRL2-alpha (short form) and PVRL2-delta (long form), both of which are ubiquitously present in various normal human tissues (Eberle, F. et al. (1995) *supra*). It is believed that the two isoforms are generated by alternative splicing from a primary transcript (Morrison, M. and Racaniello, V. (1992) J. Virol. 66: 2807-2813).

Nectin-3 (poliovirus receptor-related 3) is also a putative cell adhesion molecule that associates with afadin (Reymond, N. et al. (2000) Gene; 255(2): 347-55). Nectin3/PRR3 is a transmembrane protein, whose extracellular region contains three Ig-like domains (V, C and C) and is approximately 30% identical to other members of this family (Reymond, N. et al. (2000) *supra*). It is mainly expressed in testis and placental tissues. Nectin1, nectin2, and nectin 3 are specifically expressed at the intercellular junctions (Reymond, N. et al. (2000) *supra*).

LNIR is a protein containing three immunoglobulin (Ig) domains, may play a role in protein-protein and protein-ligand interactions, and has low similarity to poliovirus receptor-related 3 (nectin-3), which is a cell adhesion molecule (Reymond, N.et al. (2001) *supra*).

Tumor-associated glycoprotein pE4 (Tage4) is a tumor antigen and member of the immunoglobulin gene superfamily (Baury, B. et al. (2001) Gene; 265(1-2): 185-94). It has three immunoglobulin-like domains and may function in cell-cell adhesion, cell recognition, or viral entry (Baury, B. et al. (2001) *supra*). Tage4 is expressed in rat carcinoma cell Lines and upregulated in rat colon/large intestine tumors (Chadeneau, C., et al (1994) J Biol Chem 269:15601-5; Lim, Y. P., et al. (1996) Cancer Res 56:3934-40; Baury, B., et al. (2001) Gene 265:185-94).

In the central nervous system, many cell adhesion molecules are known to play a role in the establishment and remodeling of the neural circuit. Some of the cell adhesion molecules are known to be anchored to the membrane by the glycosylphosphatidylinositol (GPI) inserted to their C termini, and many GPI-anchored proteins are known to be localized in a Triton-insoluble membrane fraction of low density or so-called "raft" (Nobuo, F. et al. (1999) J Biol Chem; 274(12):8224-30).

Neurotrimin (HNT) is a GPI-anchored protein and a member of the IgLON subfamily of immunoglobulins (Struyk, A. et al. (1995) J Neurosci (3 Pt 2): 2141-56). Neurotrimin contains three immunoglobulin-like domains and is differentially expressed during development (Struyk, A. et al. (1995) *supra*). Neurotrimin is highly expressed in several developing projection systems: in neurons of the thalamus, subplate, and lower cortical laminae in the forebrain and in the pontine nucleus, cerebellar granule cells, and Purkinje cells in the hindbrain. Neurotrimin is also highly expressed in the olfactory bulb, neural retina, dorsal root ganglia, spinal cord, and in a graded distribution in the basal ganglia and hippocampus (Struyk, A. et al. (1995) *supra*).

Opioid-binding protein-cell adhesion molecule-like (OPCML or OBCAM) is a protein that binds opioid alkaloids in the presence of acidic lipids, showing selectivity for mu ligands (Shark, K. Lee, N. (1995) Gene 155: 213-217). It shares structural homology with members of the immunoglobulin protein superfamily, especially with cell-adhesion molecules. It is an extracellular molecule, and the presence of a hydrophobic C terminus suggests that it may be inserted into the cell membrane through phosphatidylinositol linkage (Shark, K. Lee, N. (1995) *supra*). Due to the lack of transmembrane domains necessary for signal transduction, it is not likely that OBCAM acts independently as an

opioid receptor; but probably plays an important accessory role in opioid receptor function (Shark, K. Lee, N. (1995) *supra*).

KIAA1867 is a protein containing five immunoglobulin (Ig) domains, which may play a role in protein-protein and protein-ligand interactions (Nagase, T. et al. (2001) DNA Res;8(2): 85-95). It has a region of low similarity to a region of nephrosis 1 which may have a role in cell-cell interactions (Nagase, T. et al. (2001) *supra*).

Limbic system-associated membrane protein (LAMP or LSAMP) is also a member of the immunoglobulin superfamily that may be involved in the function and development of the limbic system (Pimenta, A. et al. (1996) Gene 170: 189-195). During limbic development, LAMP is found on the surface of axonal membranes and growth cones, where it modulates selective homophilic adhesion molecule, and controls the development of specific patterns of neuronal connections (Pimenta, A. et al. (1996) *supra*). The gene contains a secretory signal sequence, a hydrophobic C-terminus typical of proteins linked by GPI-membrane anchors, 8 putative N-linked glycosylation sites, 3 Ig domains, and several putative phosphorylation sites.

Kilon is another GPI-anchored protein and an immunoglobulin superfamily member that may be involved in the construction and remodeling of the nervous system by facilitating rearrangement of the dendritic connectivity of magnocellular neurons (Nobuo, F. et al. (1999) *supra*). Expression of Kilon is exculsive to the brain.

The ability to manipulate the genomes of model organisms such as *Drosophila* provides a powerful means to analyze biochemical processes that, due to significant evolutionary conservation, have direct relevance to more complex vertebrate organisms. Due to a high level of gene and pathway conservation, the strong similarity of cellular processes, and the functional conservation of genes between these model organisms and mammals, identification of the involvement of novel genes in particular pathways and their functions in such model organisms can directly contribute to the understanding of the correlative pathways and methods of modulating them in mammals (see, for example, Mechler BM et al., 1985 EMBO J 4:1551-1557; Gateff E. 1982 Adv. Cancer Res. 37: 33-74; Watson KL., et al., 1994 J Cell Sci. 18: 19-33; Miklos GL, and Rubin GM. 1996 Cell 86:521-529; Wassarman DA, et al., 1995 Curr Opin Gen Dev 5: 44-50; and Booth DR. 1999 Cancer Metastasis Rev. 18: 261-284). For example, a genetic screen can be carried out in an invertebrate model organism having underexpression (e.g. knockout) or overexpression of a gene (referred to as a "genetic entry point") that yields a visible phenotype. Additional genes are mutated in a random or targeted manner. When a gene

mutation changes the original phenotype caused by the mutation in the genetic entry point, the gene is identified as a "modifier" involved in the same or overlapping pathway as the genetic entry point. When the genetic entry point is an ortholog of a human gene implicated in a disease pathway, such as p53, modifier genes can be identified that may be attractive candidate targets for novel therapeutics.

All references cited herein, including sequence information in referenced Genbank identifier numbers and website references, are incorporated herein in their entireties.

SUMMARY OF THE INVENTION

We have discovered genes that modify the p53 pathway in *Drosophila*, and identified their human orthologs, hereinafter referred to as IG. The invention provides isolated nucleic acid molecules that comprise nucleic acid sequences encoding IG protein as well as fragments and derivatives thereof. Vectors and host cells comprising the IG nucleic acid molecules are also described.

The invention provides methods for utilizing these p53 modifier genes and polypeptides to identify candidate therapeutic agents that can be used in the treatment of disorders associated with defective p53 function. Preferred IG-modulating agents specifically bind to IG polypeptides and restore p53 function. Other preferred IG-modulating agents are nucleic acid modulators such as antisense oligomers and RNAi that repress IG gene expression or product activity by, for example, binding to and inhibiting the respective nucleic acid (i.e. DNA or mRNA).

IG-specific modulating agents may be evaluated by any convenient *in vitro* or *in vivo* assay for molecular interaction with an IG polypeptide or nucleic acid. In one embodiment, candidate p53 modulating agents are tested with an assay system comprising an IG polypeptide or nucleic acid. Candidate agents that produce a change in the activity of the assay system relative to controls are identified as candidate p53 modulating agents. The assay system may be cell-based or cell-free. IG-modulating agents include IG related proteins (e.g. dominant negative mutants, and biotherapeutics); IG-specific antibodies; IG-specific antisense oligomers and other nucleic acid modulators; and chemical agents that specifically bind IG or compete with IG binding target. In one specific embodiment, a small molecule modulator is identified using a binding assay. In specific embodiments, the screening assay system is selected from a binding assay, an apoptosis assay, a cell proliferation assay, an angiogenesis assay, and a hypoxic induction assay.

In another embodiment, candidate p53 pathway modulating agents are further tested using a second assay system that detects changes in the p53 pathway, such as angiogenic, apoptotic, or cell proliferation changes produced by the originally identified candidate agent or an agent derived from the original agent. The second assay system may use cultured cells or non-human animals. In specific embodiments, the secondary assay system uses non-human animals, including animals predetermined to have a disease or disorder implicating the p53 pathway, such as an angiogenic, apoptotic, or cell proliferation disorder (e.g. cancer).

The invention further provides methods for modulating the p53 pathway in a mammalian cell by contacting the mammalian cell with an agent that specifically binds an IG polypeptide or nucleic acid. The agent may be a small molecule modulator, a nucleic acid modulator, or an antibody and may be administered to a mammalian animal predetermined to have a pathology associated the p53 pathway.

DETAILED DESCRIPTION OF THE INVENTION

Genetic screens were designed to identify modifiers of the p53 pathway in *Drosophila* in which p53 was overexpressed in the wing (Ollmann M, et al., Cell 2000 101: 91-101). The CG14372 gene was identified as a modifier of the p53 pathway. Accordingly, vertebrate orthologs of these modifiers, and preferably the human orthologs, immunoglobulin superfamily member (IG) genes (i.e., nucleic acids and polypeptides) are attractive drug targets for the treatment of pathologies associated with a defective p53 signaling pathway, such as cancer.

In vitro and in vivo methods of assessing IG function are provided herein. Modulation of the IG or their respective binding partners is useful for understanding the association of the p53 pathway and its members in normal and disease conditions and for developing diagnostics and therapeutic modalities for p53 related pathologies. IG-modulating agents that act by inhibiting or enhancing IG expression, directly or indirectly, for example, by affecting an IG function such as binding activity, can be identified using methods provided herein. IG modulating agents are useful in diagnosis, therapy and pharmaceutical development.

Nucleic acids and polypeptides of the invention

Sequences related to IG nucleic acids and polypeptides that can be used in the invention are disclosed in Genbank (referenced by Genbank identifier (GI) number) as

GI#s 12310958 (SEQ ID NO:1), 11386198 (SEQ ID NO:4), 14738423 (SEQ ID NO:5), 3451333 (SEQ ID NO:6), 20545425 (SEQ ID NO:7), 15789228 (SEQ ID NO:8), 5457320 (SEQ ID NO:11), 11056045 (SEQ ID NO:14), 15636797 (SEQ ID NO:15), 7705412 (SEQ ID NO:16), 18547571 (SEQ ID NO:20), 14017950 (SEQ ID NO:21), 16182763 (SEQ ID NO:22), 9049507 (SEQ ID NO:23), 16716338 (SEQ ID NO:26), 11067408 (SEQ ID NO:27), 4505024 (SEQ ID NO:28), 18598901 (SEQ ID NO:31), 13518022 (SEQ ID NO:32), 4505504 (SEQ ID NO:35), 11602905 (SEQ ID NO:36), 1524087 (SEQ ID NO:38), 5360209 (SEQ ID NO:41), 18589873 (SEQ ID NO:42), and 8394410 (SEQ ID NO:43) for nucleic acid, and GI#s 12310959 (SEQ ID NO:44), 11386199 (SEQ ID NO:45), 3451335 (SEQ ID NO:46), 5918159 (SEQ ID NO:49), 7705413 (SEQ ID NO:50), 14728132 (SEQ ID NO:51), 14017951 (SEQ ID NO:52), 16182764 (SEQ ID NO:53), 9049508 (SEQ ID NO:54), 16716339 (SEQ ID NO:55), 8134522 (SEQ ID NO:56), 11067409 (SEQ ID NO:57), 4505025 (SEQ ID NO:58), 4505505 (SEQ ID NO:59), 11602906 (SEQ ID NO:60), 12643789 (SEQ ID NO:61), 5360210 (SEQ ID NO:62), and 8394411 (SEQ ID NO:63) for polypeptides. Novel nucleic acid sequences of SEQ ID NOs:2, 3, 9, 10, 12, 13, 17, 18, 19, 24, 25, 29, 30, 33, 34, 37, 39, 40, and novel polypeptide sequences of SEQ ID NOs:47 and 48 can also be used in the invention. Sequence of GI#15789228 (SEQ ID NO:8) was used to deduce full length FLF22162 cDNA (SEQ ID NO:9) and polypeptide (SEQ ID NO:47), as described in Example VI.

IGs are proteins with immunoglobulin domains. The term "IG polypeptide" refers to a full-length IG protein or a functionally active fragment or derivative thereof. A "functionally active" IG fragment or derivative exhibits one or more functional activities associated with a full-length, wild-type IG protein, such as antigenic or immunogenic activity, ability to bind natural cellular substrates, etc. The functional activity of IG proteins, derivatives and fragments can be assayed by various methods known to one skilled in the art (Current Protocols in Protein Science (1998) Coligan *et al.*, eds., John Wiley & Sons, Inc., Somerset, New Jersey) and as further discussed below. For purposes herein, functionally active fragments also include those fragments that comprise one or more structural domains of an IG, such as a binding domain. Protein domains can be identified using the PFAM program (Bateman A., et al., Nucleic Acids Res, 1999, 27:260-2; http://pfam.wustl.edu). For example, the immunoglobulin domains (PFAM 00047) of IG from GI# 12310959 (SEQ ID NO:44) is located at approximately amino acid residues 46 to 115, 148 to 214, and 250 to 307. Methods for obtaining IG polypeptides are also further described below. In some embodiments, preferred fragments are functionally

active, domain-containing fragments comprising at least 25 contiguous amino acids, preferably at least 50, more preferably 75, and most preferably at least 100 contiguous amino acids of any one of SEQ ID NOs:44-63 (an IG). In further preferred embodiments, the fragment comprises the entire immunoglobulin (functionally active) domain.

IG protein derivatives typically share a certain degree of sequence identity or sequence similarity with SEQ ID NOs:47 or 48 or a fragment thereof. IG derivatives can be produced by various methods known in the art. The manipulations which result in their production can occur at the gene or protein level. For example, a cloned IG gene sequence can be cleaved at appropriate sites with restriction endonuclease(s) (Wells *et al.*, Philos. Trans. R. Soc. London SerA (1986) 317:415), followed by further enzymatic modification if desired, isolated, and ligated *in vitro*, and expressed to produce the desired derivative. Alternatively, an IG gene can be mutated *in vitro* or *in vivo*, to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions and/or to form new restriction endonuclease sites or destroy preexisting ones, to facilitate further *in vitro* modification. A variety of mutagenesis techniques are known in the art such as chemical mutagenesis, *in vitro* site-directed mutagenesis (Carter *et al.*, Nucl. Acids Res. (1986) 13:4331), use of TAB® linkers (available from Pharmacia and Upjohn, Kalamazoo, MI), *etc.*

At the protein level, manipulations include post translational modification, *e.g.* glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, *etc.* Any of numerous chemical modifications may be carried out by known technique (*e.g.* specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, $NaBH_4$, acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin, *etc.*). Derivative proteins can also be chemically synthesized by use of a peptide synthesizer, for example to introduce nonclassical amino acids or chemical amino acid analogs as substitutions or additions into the IG protein sequence.

Chimeric or fusion proteins can be made comprising an IG protein or fragment thereof (preferably comprising one or more structural or functional domains of the IG protein) joined at its amino- or carboxy-terminus via a peptide bond to an amino acid sequence of a different protein. Chimeric proteins can be produced by any known method, including: recombinant expression of a nucleic acid encoding the protein (comprising a IG-coding sequence joined in-frame to a coding sequence for a different protein); ligating the

appropriate nucleic acid sequences encoding the desired amino acid sequences to each other in the proper coding frame, and expressing the chimeric product; and protein synthetic techniques, *e.g.* by use of a peptide synthesizer.

The subject IG polypeptides also encompass minor deletion mutants, including N-, and/or C-terminal truncations. Such deletion mutants are readily screened for IG competitive or dominant negative activity.

The term "IG nucleic acid" refers to a DNA or RNA molecule that encodes an IG polypeptide. In preferred embodiments, the nucleic acid encodes a polypeptide selected from the group consisting of SEQ ID NOs:47 and 48. In some embodiments, the nucleic acid comprises a sequence selected from the group consisting of SEQ ID NOs:9 and 10. In a specific embodiment, the invention provides an isolated nucleic acid which encodes a human IG as shown in SEQ ID NOs:47 or 48.

The invention includes a fragment of a nucleic acid, such as a fragment that encodes a binding domain of one of the full-length sequences of the invention. Fragments of an IG nucleic acid sequence can be used for a variety of purposes. As an example, interfering RNA (RNAi) fragments, particularly double-stranded (ds) RNAi, can be used to generate loss-of-function phenotypes; which can, in turn, be used, among other uses, to determine gene function. Certain "antisense" fragments, i.e. that are reverse complements of portions of the coding and/or untranslated regions (e.g. 5' UTR) have utility in inhibiting the function of IG proteins. The fragments are of length sufficient to specifically hybridize with the corresponding IG sequence. The fragments consist of or comprise at least 12, preferably at least 24, more preferably at least 36, and more preferably at least 96 contiguous nucleotides of IG. When the fragments are flanked by other nucleic acid sequences, the total length of the combined nucleic acid sequence is less than 15 kb, preferably less than 10 kb or less than 5kb, more preferably less than 2 kb, and in some cases, preferably less than 500 bases.

In other specific embodiments, preferred fragments of SEQ ID NO:9 encode extracellular or intracellular domains which are located at approximately nucleotides 3-999 and 1059-1167. Additional preferred fragments of SEQ ID NO:9 encode Immunoglobulin domains which are located approximately at nucleotides 90-366, 393-666, and 693-930. These domains may be useful to locate the function and/or binding partners of a protein. For example, a nucleic acid that encodes an extracellular or intracellular domain of a protein may be used to screen for binding partners related to the protein.

The subject nucleic acid sequences may consist solely of the IG nucleic acid or fragments thereof. Alternatively, the subject nucleic acid sequences and fragments thereof may be joined to other components such as labels, peptides, agents that facilitate transport across cell membranes, hybridization-triggered cleavage agents or intercalating agents. The subject nucleic acid sequences and fragments thereof may also be joined to other nucleic acid sequences (i.e. they may comprise part of larger sequences) and are of synthetic/non-natural sequences and/or are isolated and/or are purified, i.e. unaccompanied by at least some of the material with which it is associated in its natural state. Preferably, the isolated nucleic acids constitute at least about 0.5%, and more preferably at least about 5% by weight of the total nucleic acid present in a given fraction, and are preferably recombinant, meaning that they comprise a non-natural sequence or a natural sequence joined to nucleotide(s) other than that which it is joined to on a natural chromosome.

The subject nucleic acids find a wide variety of applications including use as translatable transcripts, hybridization probes, PCR primers, diagnostic nucleic acids, etc.; use in detecting the presence of IG genes and gene transcripts and in detecting or amplifying nucleic acids encoding additional IG homologs and structural analogs. In diagnosis, IG hybridization probes find use in identifying wild-type and mutant IG alleles in clinical and laboratory samples. Mutant alleles are used to generate allele-specific oligonucleotide (ASO) probes for high-throughput clinical diagnoses. In therapy, therapeutic IG nucleic acids are used to modulate cellular expression or intracellular concentration or availability of active IG.

In one preferred embodiment, the derivative nucleic acid encodes a polypeptide comprising an IG amino acid sequence of SEQ ID NOs:47 or 48, or a fragment or derivative thereof. A derivative IG nucleic acid sequence, or fragment thereof, may comprise 100% sequence identity with SEQ ID NOs:9 or 10, but be a derivative thereof in the sense that it has one or more modifications at the base or sugar moiety, or phosphate backbone. Examples of modifications are well known in the art (Bailey, Ullmann's Encyclopedia of Industrial Chemistry (1998), 6th ed. Wiley and Sons). Such derivatives may be used to provide modified stability or any other desired property.

Preferably, the IG polypeptide or nucleic acid or fragment thereof is from a human, but can also be an ortholog, or derivative thereof with at least 70% sequence identity, preferably at least 80%, more preferably 85%, still more preferably 90%, and most preferably at least 95% sequence identity with IG. Normally, orthologs in different species retain the same function, due to presence of one or more protein motifs and/or 3-

dimensional structures. Orthologs are generally identified by sequence homology analysis, such as BLAST analysis, usually using protein bait sequences. Sequences are assigned as a potential ortholog if the best hit sequence from the forward BLAST result retrieves the original query sequence in the reverse BLAST (Huynen MA and Bork P, Proc Natl Acad Sci (1998) 95:5849-5856; Huynen MA *et al.*, Genome Research (2000) 10:1204-1210). Programs for multiple sequence alignment, such as CLUSTAL (Thompson JD et al, 1994, Nucleic Acids Res 22:4673-4680) may be used to highlight conserved regions and/or residues of orthologous proteins and to generate phylogenetic trees. In a phylogenetic tree representing multiple homologous sequences from diverse species (e.g., retrieved through BLAST analysis), orthologous sequences from two species generally appear closest on the tree with respect to all other sequences from these two species. Structural threading or other analysis of protein folding (e.g., using software by ProCeryon, Biosciences, Salzburg, Austria) may also identify potential orthologs. In evolution, when a gene duplication event follows speciation, a single gene in one species, such as *Drosophila*, may correspond to multiple genes (paralogs) in another, such as human. As used herein, the term "orthologs" encompasses paralogs. As used herein, "percent (%) sequence identity" with respect to a subject sequence, or a specified portion of a subject sequence, is defined as the percentage of nucleotides or amino acids in the candidate derivative sequence identical with the nucleotides or amino acids in the subject sequence (or specified portion thereof), after aligning the sequences and introducing gaps, if necessary to achieve the maximum percent sequence identity, as generated by the program WU-BLAST-2.0a19 (Altschul *et al.*, J. Mol. Biol. (1997) 215:403-410; http://blast.wustl.edu/blast/README.html) with all the search parameters set to default values. The HSP S and HSP S2 parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched. A % identity value is determined by the number of matching identical nucleotides or amino acids divided by the sequence length for which the percent identity is being reported. "Percent (%) amino acid sequence similarity" is determined by doing the same calculation as for determining % amino acid sequence identity, but including conservative amino acid substitutions in addition to identical amino acids in the computation.

A conservative amino acid substitution is one in which an amino acid is substituted for another amino acid having similar properties such that the folding or activity of the protein is not significantly affected. Aromatic amino acids that can be substituted for each other

are phenylalanine, tryptophan, and tyrosine; interchangeable hydrophobic amino acids are leucine, isoleucine, methionine, and valine; interchangeable polar amino acids are glutamine and asparagine; interchangeable basic amino acids are arginine, lysine and histidine; interchangeable acidic amino acids are aspartic acid and glutamic acid; and interchangeable small amino acids are alanine, serine, threonine, cysteine and glycine.

Alternatively, an alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman (Smith and Waterman, 1981, Advances in Applied Mathematics 2:482-489; database: European Bioinformatics Institute http://www.ebi.ac.uk/MPsrch/; Smith and Waterman, 1981, J. of Molec.Biol., 147:195-197; Nicholas et al., 1998, "A Tutorial on Searching Sequence Databases and Sequence Scoring Methods" (www.psc.edu) and references cited therein.; W.R. Pearson, 1991, Genomics 11:635-650). This algorithm can be applied to amino acid sequences by using the scoring matrix developed by Dayhoff (Dayhoff: Atlas of Protein Sequences and Structure, M. O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA), and normalized by Gribskov (Gribskov 1986 Nucl. Acids Res. 14(6):6745-6763). The Smith-Waterman algorithm may be employed where default parameters are used for scoring (for example, gap open penalty of 12, gap extension penalty of two). From the data generated, the "Match" value reflects "sequence identity."

Derivative nucleic acid molecules of the subject nucleic acid molecules include sequences that hybridize to the nucleic acid sequence of any of SEQ ID NOs:1-43. The stringency of hybridization can be controlled by temperature, ionic strength, pH, and the presence of denaturing agents such as formamide during hybridization and washing. Conditions routinely used are set out in readily available procedure texts (*e.g.*, Current Protocol in Molecular Biology, Vol. 1, Chap. 2.10, John Wiley & Sons, Publishers (1994); Sambrook *et al.*, Molecular Cloning, Cold Spring Harbor (1989)). In some embodiments, a nucleic acid molecule of the invention is capable of hybridizing to a nucleic acid molecule containing the nucleotide sequence of any one of SEQ ID NOs:1 - 43 under stringent hybridization conditions that comprise: prehybridization of filters containing nucleic acid for 8 hours to overnight at 65° C in a solution comprising 6X single strength citrate (SSC) (1X SSC is 0.15 M NaCl, 0.015 M Na citrate; pH 7.0), 5X Denhardt's solution, 0.05% sodium pyrophosphate and 100 μg/ml herring sperm DNA; hybridization for 18-20 hours at 65° C in a solution containing 6X SSC, 1X Denhardt's solution, 100

μg/ml yeast tRNA and 0.05% sodium pyrophosphate; and washing of filters at 65° C for 1h in a solution containing 0.2X SSC and 0.1% SDS (sodium dodecyl sulfate).

In other embodiments, moderately stringent hybridization conditions are used that comprise: pretreatment of filters containing nucleic acid for 6 h at 40° C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH7.5), 5mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 μg/ml denatured salmon sperm DNA; hybridization for 18-20h at 40° C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH7.5), 5mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 μg/ml salmon sperm DNA, and 10% (wt/vol) dextran sulfate; followed by washing twice for 1 hour at 55° C in a solution containing 2X SSC and 0.1% SDS.

Alternatively, low stringency conditions can be used that comprise: incubation for 8 hours to overnight at 37° C in a solution comprising 20% formamide, 5 x SSC, 50 mM sodium phosphate (pH 7.6), 5X Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured sheared salmon sperm DNA; hybridization in the same buffer for 18 to 20 hours; and washing of filters in 1 x SSC at about 37° C for 1 hour.

Isolation, Production, Expression, and Mis-expression of IG Nucleic Acids and Polypeptides

IG nucleic acids and polypeptides, useful for identifying and testing agents that modulate IG function and for other applications related to the involvement of IG in the p53 pathway. IG nucleic acids and derivatives and orthologs thereof may be obtained using any available method. For instance, techniques for isolating cDNA or genomic DNA sequences of interest by screening DNA libraries or by using polymerase chain reaction (PCR) are well known in the art. In general, the particular use for the protein will dictate the particulars of expression, production, and purification methods. For instance, production of proteins for use in screening for modulating agents may require methods that preserve specific biological activities of these proteins, whereas production of proteins for antibody generation may require structural integrity of particular epitopes. Expression of proteins to be purified for screening or antibody production may require the addition of specific tags (*e.g.*, generation of fusion proteins). Overexpression of an IG protein for assays used to assess IG function, such as involvement in cell cycle regulation or hypoxic response, may require expression in eukaryotic cell lines capable of these cellular activities. Techniques for the expression, production, and purification of proteins are well known in the art; any suitable means therefore may be used (e.g., Higgins SJ and Hames

BD (eds.) Protein Expression: A Practical Approach, Oxford University Press Inc., New York 1999; Stanbury PF et al., Principles of Fermentation Technology, 2nd edition, Elsevier Science, New York, 1995; Doonan S (ed.) Protein Purification Protocols, Humana Press, New Jersey, 1996; Coligan JE et al, Current Protocols in Protein Science (eds.), 1999, John Wiley & Sons, New York). In particular embodiments, recombinant IG is expressed in a cell line known to have defective p53 function (e.g. SAOS-2 osteoblasts, H1299 lung cancer cells, C33A and HT3 cervical cancer cells, HT-29 and DLD-1 colon cancer cells, among others, available from American Type Culture Collection (ATCC), Manassas, VA). The recombinant cells are used in cell-based screening assay systems of the invention, as described further below.

The nucleotide sequence encoding an IG polypeptide can be inserted into any appropriate expression vector. The necessary transcriptional and translational signals, including promoter/enhancer element, can derive from the native IG gene and/or its flanking regions or can be heterologous. A variety of host-vector expression systems may be utilized, such as mammalian cell systems infected with virus (*e.g.* vaccinia virus, adenovirus, *etc.*); insect cell systems infected with virus (*e.g.* baculovirus); microorganisms such as yeast containing yeast vectors, or bacteria transformed with bacteriophage, plasmid, or cosmid DNA. A host cell strain that modulates the expression of, modifies, and/or specifically processes the gene product may be used.

To detect expression of the IG gene product, the expression vector can comprise a promoter operably linked to an IG gene nucleic acid, one or more origins of replication, and, one or more selectable markers (*e.g.* thymidine kinase activity, resistance to antibiotics, *etc.*). Alternatively, recombinant expression vectors can be identified by assaying for the expression of the IG gene product based on the physical or functional properties of the IG protein in *in vitro* assay systems (*e.g.* immunoassays).

The IG protein, fragment, or derivative may be optionally expressed as a fusion, or chimeric protein product (i.e. it is joined via a peptide bond to a heterologous protein sequence of a different protein), for example to facilitate purification or detection. A chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other using standard methods and expressing the chimeric product. A chimeric product may also be made by protein synthetic techniques, *e.g.* by use of a peptide synthesizer (Hunkapiller *et al.*, Nature (1984) 310:105-111).

Once a recombinant cell that expresses the IG gene sequence is identified, the gene product can be isolated and purified using standard methods (*e.g.* ion exchange, affinity,

and gel exclusion chromatography; centrifugation; differential solubility; electrophoresis, cite purification reference). Alternatively, native IG proteins can be purified from natural sources, by standard methods (*e.g.* immunoaffinity purification). Once a protein is obtained, it may be quantified and its activity measured by appropriate methods, such as immunoassay, bioassay, or other measurements of physical properties, such as crystallography.

The methods of this invention may also use cells that have been engineered for altered expression (mis-expression) of IG or other genes associated with the p53 pathway. As used herein, mis-expression encompasses ectopic expression, over-expression, under-expression, and non-expression (*e.g.* by gene knock-out or blocking expression that would otherwise normally occur).

Genetically modified animals

Animal models that have been genetically modified to alter IG expression may be used in *in vivo* assays to test for activity of a candidate p53 modulating agent, or to further assess the role of IG in a p53 pathway process such as apoptosis or cell proliferation. Preferably, the altered IG expression results in a detectable phenotype, such as decreased or increased levels of cell proliferation, angiogenesis, or apoptosis compared to control animals having normal IG expression. The genetically modified animal may additionally have altered p53 expression (e.g. p53 knockout). Preferred genetically modified animals are mammals such as primates, rodents (preferably mice), cows, horses, goats, sheep, pigs, dogs and cats. Preferred non-mammalian species include zebrafish, *C. elegans*, and *Drosophila*. Preferred genetically modified animals are transgenic animals having a heterologous nucleic acid sequence present as an extrachromosomal element in a portion of its cells, i.e. mosaic animals (see, for example, techniques described by Jakobovits, 1994, Curr. Biol. 4:761-763.) or stably integrated into its germ line DNA (i.e., in the genomic sequence of most or all of its cells). Heterologous nucleic acid is introduced into the germ line of such transgenic animals by genetic manipulation of, for example, embryos or embryonic stem cells of the host animal.

Methods of making transgenic animals are well-known in the art (for transgenic mice see Brinster et al., Proc. Nat. Acad. Sci. USA 82: 4438-4442 (1985), U.S. Pat. Nos. 4,736,866 and 4,870,009, both by Leder et al., U.S. Pat. No. 4,873,191 by Wagner et al., and Hogan, B., Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1986); for particle bombardment see U.S. Pat. No., 4,945,050,

by Sandford *et al.*; for transgenic *Drosophila* see Rubin and Spradling, Science (1982) 218:348-53 and U.S. Pat. No. 4,670,388; for transgenic insects see Berghammer A.J. *et al.*, A Universal Marker for Transgenic Insects (1999) Nature 402:370-371; for transgenic Zebrafish see Lin S., Transgenic Zebrafish, Methods Mol Biol. (2000);136:375-3830); for microinjection procedures for fish, amphibian eggs and birds see Houdebine and Chourrout, Experientia (1991) 47:897-905; for transgenic rats see Hammer *et al.*, Cell (1990) 63:1099-1112; and for culturing of embryonic stem (ES) cells and the subsequent production of transgenic animals by the introduction of DNA into ES cells using methods such as electroporation, calcium phosphate/DNA precipitation and direct injection see, e.g., Teratocarcinomas and Embryonic Stem Cells, A Practical Approach, E. J. Robertson, ed., IRL Press (1987)). Clones of the nonhuman transgenic animals can be produced according to available methods (see Wilmut, I. *et al.* (1997) Nature 385:810-813; and PCT International Publication Nos. WO 97/07668 and WO 97/07669).

In one embodiment, the transgenic animal is a "knock-out" animal having a heterozygous or homozygous alteration in the sequence of an endogenous IG gene that results in a decrease of IG function, preferably such that IG expression is undetectable or insignificant. Knock-out animals are typically generated by homologous recombination with a vector comprising a transgene having at least a portion of the gene to be knocked out. Typically a deletion, addition or substitution has been introduced into the transgene to functionally disrupt it. The transgene can be a human gene (e.g., from a human genomic clone) but more preferably is an ortholog of the human gene derived from the transgenic host species. For example, a mouse IG gene is used to construct a homologous recombination vector suitable for altering an endogenous IG gene in the mouse genome. Detailed methodologies for homologous recombination in mice are available (see Capecchi, Science (1989) 244:1288-1292; Joyner *et al.*, Nature (1989) 338:153-156). Procedures for the production of non-rodent transgenic mammals and other animals are also available (Houdebine and Chourrout, *supra*; Pursel *et al.*, Science (1989) 244:1281-1288; Simms *et al.*, Bio/Technology (1988) 6:179-183). In a preferred embodiment, knock-out animals, such as mice harboring a knockout of a specific gene, may be used to produce antibodies against the human counterpart of the gene that has been knocked out (Claesson MH et al., (1994) Scan J Immunol 40:257-264; Declerck PJ et al., (1995) J Biol Chem. 270:8397-400).

In another embodiment, the transgenic animal is a "knock-in" animal having an alteration in its genome that results in altered expression (e.g., increased (including

ectopic) or decreased expression) of the IG gene, e.g., by introduction of additional copies of IG, or by operatively inserting a regulatory sequence that provides for altered expression of an endogenous copy of the IG gene. Such regulatory sequences include inducible, tissue-specific, and constitutive promoters and enhancer elements. The knock-in can be homozygous or heterozygous.

Transgenic nonhuman animals can also be produced that contain selected systems allowing for regulated expression of the transgene. One example of such a system that may be produced is the cre/loxP recombinase system of bacteriophage P1 (Lakso *et al.*, PNAS (1992) 89:6232-6236; U.S. Pat. No. 4,959,317). If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase. Another example of a recombinase system is the FLP recombinase system of Saccharomyces cerevisiae (O'Gorman et al. (1991) Science 251:1351-1355; U.S. Pat. No. 5,654,182). In a preferred embodiment, both Cre-LoxP and Flp-Frt are used in the same system to regulate expression of the transgene, and for sequential deletion of vector sequences in the same cell (Sun X et al (2000) Nat Genet 25:83-6).

The genetically modified animals can be used in genetic studies to further elucidate the p53 pathway, as animal models of disease and disorders implicating defective p53 function, and for *in vivo* testing of candidate therapeutic agents, such as those identified in screens described below. The candidate therapeutic agents are administered to a genetically modified animal having altered IG function and phenotypic changes are compared with appropriate control animals such as genetically modified animals that receive placebo treatment, and/or animals with unaltered IG expression that receive candidate therapeutic agent.

In addition to the above-described genetically modified animals having altered IG function, animal models having defective p53 function (and otherwise normal IG function), can be used in the methods of the present invention. For example, a p53 knockout mouse can be used to assess, *in vivo*, the activity of a candidate p53 modulating agent identified in one of the *in vitro* assays described below. p53 knockout mice are described in the literature (Jacks et al., Nature 2001;410:1111-1116, 1043-1044; Donehower *et al.*, supra). Preferably, the candidate p53 modulating agent when

administered to a model system with cells defective in p53 function, produces a detectable phenotypic change in the model system indicating that the p53 function is restored, i.e., the cells exhibit normal cell cycle progression.

Modulating Agents

The invention provides methods to identify agents that interact with and/or modulate the function of IG and/or the p53 pathway. Such agents are useful in a variety of diagnostic and therapeutic applications associated with the p53 pathway, as well as in further analysis of the IG protein and its contribution to the p53 pathway. Accordingly, the invention also provides methods for modulating the p53 pathway comprising the step of specifically modulating IG activity by administering an IG-interacting or -modulating agent.

In a preferred embodiment, IG-modulating agents inhibit or enhance IG activity or otherwise affect normal IG function, including transcription, protein expression, protein localization, and cellular or extra-cellular activity. In a further preferred embodiment, the candidate p53 pathway- modulating agent specifically modulates the function of the IG. The phrases "specific modulating agent", "specifically modulates", etc., are used herein to refer to modulating agents that directly bind to the IG polypeptide or nucleic acid, and preferably inhibit, enhance, or otherwise alter, the function of the IG. The term also encompasses modulating agents that alter the interaction of the IG with a binding partner or substrate (e.g. by binding to a binding partner of an IG, or to a protein/binding partner complex, and inhibiting function).

Preferred IG-modulating agents include small molecule compounds; IG-interacting proteins, including antibodies and other biotherapeutics; and nucleic acid modulators such as antisense and RNA inhibitors. The modulating agents may be formulated in pharmaceutical compositions, for example, as compositions that may comprise other active ingredients, as in combination therapy, and/or suitable carriers or excipients. Techniques for formulation and administration of the compounds may be found in "Remington's Pharmaceutical Sciences" Mack Publishing Co., Easton, PA, 19th edition.

Small molecule modulators

Small molecules, are often preferred to modulate function of proteins with enzymatic function, and/or containing protein interaction domains. Chemical agents, referred to in the art as "small molecule" compounds are typically organic, non-peptide molecules,

having a molecular weight less than 10,000, preferably less than 5,000, more preferably less than 1,000, and most preferably less than 500. This class of modulators includes chemically synthesized molecules, for instance, compounds from combinatorial chemical libraries. Synthetic compounds may be rationally designed or identified based on known or inferred properties of the IG protein or may be identified by screening compound libraries. Alternative appropriate modulators of this class are natural products, particularly secondary metabolites from organisms such as plants or fungi, which can also be identified by screening compound libraries for IG–modulating activity. Methods for generating and obtaining compounds are well known in the art (Schreiber SL, Science (2000) 151: 1964-1969; Radmann J and Gunther J, Science (2000) 151:1947-1948).

Small molecule modulators identified from screening assays, as described below, can be used as lead compounds from which candidate clinical compounds may be designed, optimized, and synthesized. Such clinical compounds may have utility in treating pathologies associated with the p53 pathway. The activity of candidate small molecule modulating agents may be improved several-fold through iterative secondary functional validation, as further described below, structure determination, and candidate modulator modification and testing. Additionally, candidate clinical compounds are generated with specific regard to clinical and pharmacological properties. For example, the reagents may be derivatized and re-screened using *in vitro* and *in vivo* assays to optimize activity and minimize toxicity for pharmaceutical development.

Protein Modulators

Specific IG-interacting proteins are useful in a variety of diagnostic and therapeutic applications related to the p53 pathway and related disorders, as well as in validation assays for other IG-modulating agents. In a preferred embodiment, IG-interacting proteins affect normal IG function, including transcription, protein expression, protein localization, and cellular or extra-cellular activity. In another embodiment, IG-interacting proteins are useful in detecting and providing information about the function of IG proteins, as is relevant to p53 related disorders, such as cancer (e.g., for diagnostic means).

An IG-interacting protein may be endogenous, i.e. one that naturally interacts genetically or biochemically with an IG, such as a member of the IG pathway that modulates IG expression, localization, and/or activity. IG-modulators include dominant negative forms of IG-interacting proteins and of IG proteins themselves. Yeast two-hybrid and variant screens offer preferred methods for identifying endogenous IG-interacting

proteins (Finley, R. L. et al. (1996) in DNA Cloning-Expression Systems: A Practical Approach, eds. Glover D. & Hames B. D (Oxford University Press, Oxford, England), pp. 169-203; Fashema SF et al., Gene (2000) 250:1-14; Drees BL Curr Opin Chem Biol (1999) 3:64-70; Vidal M and Legrain P Nucleic Acids Res (1999) 27:919-29; and U.S. Pat. No. 5,928,868). Mass spectrometry is an alternative preferred method for the elucidation of protein complexes (reviewed in, e.g., Pandley A and Mann M, Nature (2000) 405:837-846; Yates JR 3rd, Trends Genet (2000) 16:5-8).

An IG-interacting protein may be an exogenous protein, such as an IG-specific antibody or a T-cell antigen receptor (see, e.g., Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory; Harlow and Lane (1999) Using antibodies: a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press). IG antibodies are further discussed below.

In preferred embodiments, an IG-interacting protein specifically binds an IG protein. In alternative preferred embodiments, an IG-modulating agent binds an IG substrate, binding partner, or cofactor.

Antibodies

In another embodiment, the protein modulator is an IG specific antibody agonist or antagonist. The antibodies have therapeutic and diagnostic utilities, and can be used in screening assays to identify IG modulators. The antibodies can also be used in dissecting the portions of the IG pathway responsible for various cellular responses and in the general processing and maturation of the IG.

Antibodies that specifically bind IG polypeptides can be generated using known methods. Preferably the antibody is specific to a mammalian ortholog of IG polypeptide, and more preferably, to human IG. Antibodies may be polyclonal, monoclonal (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab').sub.2 fragments, fragments produced by a FAb expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. Epitopes of IG which are particularly antigenic can be selected, for example, by routine screening of IG polypeptides for antigenicity or by applying a theoretical method for selecting antigenic regions of a protein (Hopp and Wood (1981), Proc. Nati. Acad. Sci. U.S.A. 78:3824-28; Hopp and Wood, (1983) Mol. Immunol. 20:483-89; Sutcliffe et al., (1983) Science 219:660-66) to the amino acid sequence shown in any of SEQ ID NOs:44 - 63. Monoclonal antibodies with affinities of 10^8 M^{-1} preferably 10^9 M^{-1} to 10^{10} M^{-1}, or

stronger can be made by standard procedures as described (Harlow and Lane, *supra*; Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed) Academic Press, New York; and U.S. Pat. Nos. 4,381,292; 4,451,570; and 4,618,577). Antibodies may be generated against crude cell extracts of IG or substantially purified fragments thereof. If IG fragments are used, they preferably comprise at least 10, and more preferably, at least 20 contiguous amino acids of an IG protein. In a particular embodiment, IG-specific antigens and/or immunogens are coupled to carrier proteins that stimulate the immune response. For example, the subject polypeptides are covalently coupled to the keyhole limpet hemocyanin (KLH) carrier, and the conjugate is emulsified in Freund's complete adjuvant, which enhances the immune response. An appropriate immune system such as a laboratory rabbit or mouse is immunized according to conventional protocols.

The presence of IG-specific antibodies is assayed by an appropriate assay such as a solid phase enzyme-linked immunosorbant assay (ELISA) using immobilized corresponding IG polypeptides. Other assays, such as radioimmunoassays or fluorescent assays might also be used.

Chimeric antibodies specific to IG polypeptides can be made that contain different portions from different animal species. For instance, a human immunoglobulin constant region may be linked to a variable region of a murine mAb, such that the antibody derives its biological activity from the human antibody, and its binding specificity from the murine fragment. Chimeric antibodies are produced by splicing together genes that encode the appropriate regions from each species (Morrison et al., Proc. Natl. Acad. Sci. (1984) 81:6851-6855; Neuberger et al., Nature (1984) 312:604-608; Takeda et al., Nature (1985) 31:452-454). Humanized antibodies, which are a form of chimeric antibodies, can be generated by grafting complementary-determining regions (CDRs) (Carlos, T. M., J. M. Harlan. 1994. Blood 84:2068-2101) of mouse antibodies into a background of human framework regions and constant regions by recombinant DNA technology (Riechmann LM, et al., 1988 Nature 323: 323-327). Humanized antibodies contain ~10% murine sequences and ~90% human sequences, and thus further reduce or eliminate immunogenicity, while retaining the antibody specificities (Co MS, and Queen C. 1991 Nature 351: 501-501; Morrison SL. 1992 Ann. Rev. Immun. 10:239-265). Humanized antibodies and methods of their production are well-known in the art (U.S. Pat. Nos. 5,530,101, 5,585,089, 5,693,762, and 6,180,370).

IG-specific single chain antibodies which are recombinant, single chain polypeptides formed by linking the heavy and light chain fragments of the Fv regions via an amino acid

bridge, can be produced by methods known in the art (U.S. Pat. No. 4,946,778; Bird, Science (1988) 242:423-426; Huston et al., Proc. Natl. Acad. Sci. USA (1988) 85:5879-5883; and Ward et al., Nature (1989) 334:544-546).

Other suitable techniques for antibody production involve in vitro exposure of lymphocytes to the antigenic polypeptides or alternatively to selection of libraries of antibodies in phage or similar vectors (Huse et al., Science (1989) 246:1275-1281). As used herein, T-cell antigen receptors are included within the scope of antibody modulators (Harlow and Lane, 1988, *supra*).

The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, antibodies will be labeled by joining, either covalently or non-covalently, a substance that provides for a detectable signal, or that is toxic to cells that express the targeted protein (Menard S, et al., Int J. Biol Markers (1989) 4:131-134). A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, fluorescent emitting lanthanide metals, chemiluminescent moieties, bioluminescent moieties, magnetic particles, and the like (U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241). Also, recombinant immunoglobulins may be produced (U.S. Pat. No. 4,816,567). Antibodies to cytoplasmic polypeptides may be delivered and reach their targets by conjugation with membrane-penetrating toxin proteins (U.S. Pat. No. 6,086,900).

When used therapeutically in a patient, the antibodies of the subject invention are typically administered parenterally, when possible at the target site, or intravenously. The therapeutically effective dose and dosage regimen is determined by clinical studies. Typically, the amount of antibody administered is in the range of about 0.1 mg/kg –to about 10 mg/kg of patient weight. For parenteral administration, the antibodies are formulated in a unit dosage injectable form (e.g., solution, suspension, emulsion) in association with a pharmaceutically acceptable vehicle. Such vehicles are inherently nontoxic and non-therapeutic. Examples are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Nonaqueous vehicles such as fixed oils, ethyl oleate, or liposome carriers may also be used. The vehicle may contain minor amounts of additives, such as buffers and preservatives, which enhance isotonicity and chemical stability or otherwise enhance therapeutic potential. The antibodies' concentrations in such vehicles are typically in the range of about 1 mg/ml to about10 mg/ml.

Immunotherapeutic methods are further described in the literature (US Pat. No. 5,859,206; WO0073469).

Specific biotherapeutics

In a preferred embodiment, an IG-interacting protein may have biotherapeutic applications. Biotherapeutic agents formulated in pharmaceutically acceptable carriers and dosages may be used to activate or inhibit signal transduction pathways. This modulation may be accomplished by binding a ligand, thus inhibiting the activity of the pathway; or by binding a receptor, either to inhibit activation of, or to activate, the receptor. Alternatively, the biotherapeutic may itself be a ligand capable of activating or inhibiting a receptor. Biotherapeutic agents and methods of producing them are described in detail in U.S. Pat. No. 6,146,628.

IG ligand(s), antibodies to the ligand(s) or the IG itself may be used as biotherapeutics to modulate the activity of IG in the p53 pathway.

Nucleic Acid Modulators

Other preferred IG-modulating agents comprise nucleic acid molecules, such as antisense oligomers or double stranded RNA (dsRNA), which generally inhibit IG activity. Preferred nucleic acid modulators interfere with the function of the IG nucleic acid such as DNA replication, transcription, translocation of the IG RNA to the site of protein translation, translation of protein from the IG RNA, splicing of the IG RNA to yield one or more mRNA species, or catalytic activity which may be engaged in or facilitated by the IG RNA.

In one embodiment, the antisense oligomer is an oligonucleotide that is sufficiently complementary to an IG mRNA to bind to and prevent translation, preferably by binding to the 5′ untranslated region. IG-specific antisense oligonucleotides, preferably range from at least 6 to about 200 nucleotides. In some embodiments the oligonucleotide is preferably at least 10, 15, or 20 nucleotides in length. In other embodiments, the oligonucleotide is preferably less than 50, 40, or 30 nucleotides in length. The oligonucleotide can be DNA or RNA or a chimeric mixture or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone. The oligonucleotide may include other appending groups such as peptides, agents that facilitate transport across the cell membrane, hybridization-triggered cleavage agents, and intercalating agents.

In another embodiment, the antisense oligomer is a phosphothioate morpholino oligomer (PMO). PMOs are assembled from four different morpholino subunits, each of which contain one of four genetic bases (A, C, G, or T) linked to a six-membered morpholine ring. Polymers of these subunits are joined by non-ionic phosphodiamidate intersubunit linkages. Details of how to make and use PMOs and other antisense oligomers are well known in the art (e.g. see WO99/18193; Probst JC, Antisense Oligodeoxynucleotide and Ribozyme Design, Methods. (2000) 22(3):271-281; Summerton J, and Weller D. 1997 Antisense Nucleic Acid Drug Dev. :7:187-95; US Pat. No. 5,235,033; and US Pat No. 5,378,841).

Alternative preferred IG nucleic acid modulators are double-stranded RNA species mediating RNA interference (RNAi). RNAi is the process of sequence-specific, post-transcriptional gene silencing in animals and plants, initiated by double-stranded RNA (dsRNA) that is homologous in sequence to the silenced gene. Methods relating to the use of RNAi to silence genes in *C. elegans*, *Drosophila*, plants, and humans are known in the art (Fire A, et al., 1998 Nature 391:806-811; Fire, A. Trends Genet. 15, 358-363 (1999); Sharp, P. A. RNA interference 2001. Genes Dev. 15, 485-490 (2001); Hammond, S. M., et al., Nature Rev. Genet. 2, 110-1119 (2001); Tuschl, T. Chem. Biochem. 2, 239-245 (2001); Hamilton, A. et al., Science 286, 950-952 (1999); Hammond, S. M., et al., Nature 404, 293-296 (2000); Zamore, P. D., et al., Cell 101, 25-33 (2000); Bernstein, E., et al., Nature 409, 363-366 (2001); Elbashir, S. M., et al., Genes Dev. 15, 188-200 (2001); WO0129058; WO9932619; Elbashir SM, et al., 2001 Nature 411:494-498).

Nucleic acid modulators are commonly used as research reagents, diagnostics, and therapeutics. For example, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often used to elucidate the function of particular genes (see, for example, U.S. Pat. No. 6,165,790). Nucleic acid modulators are also used, for example, to distinguish between functions of various members of a biological pathway. For example, antisense oligomers have been employed as therapeutic moieties in the treatment of disease states in animals and man and have been demonstrated in numerous clinical trials to be safe and effective (Milligan JF, *et al*, Current Concepts in Antisense Drug Design, J Med Chem. (1993) 36:1923-1937; Tonkinson JL *et al.*, Antisense Oligodeoxynucleotides as Clinical Therapeutic Agents, Cancer Invest. (1996) 14:54-65). Accordingly, in one aspect of the invention, an IG-specific nucleic acid modulator is used in an assay to further elucidate the role of the IG in the p53 pathway, and/or its relationship to other members of the pathway. In another aspect of the invention, an IG-

specific antisense oligomer is used as a therapeutic agent for treatment of p53-related disease states.

Assay Systems

The invention provides assay systems and screening methods for identifying specific modulators of IG activity. As used herein, an "assay system" encompasses all the components required for performing and analyzing results of an assay that detects and/or measures a particular event. In general, primary assays are used to identify or confirm a modulator's specific biochemical or molecular effect with respect to the IG nucleic acid or protein. In general, secondary assays further assess the activity of an IG modulating agent identified by a primary assay and may confirm that the modulating agent affects IG in a manner relevant to the p53 pathway. In some cases, IG modulators will be directly tested in a secondary assay.

In a preferred embodiment, the screening method comprises contacting a suitable assay system comprising an IG polypeptide with a candidate agent under conditions whereby, but for the presence of the agent, the system provides a reference activity (e.g. binding activity), which is based on the particular molecular event the screening method detects. A statistically significant difference between the agent-biased activity and the reference activity indicates that the candidate agent modulates IG activity, and hence the p53 pathway.

Primary Assays

The type of modulator tested generally determines the type of primary assay.

Primary assays for small molecule modulators

For small molecule modulators, screening assays are used to identify candidate modulators. Screening assays may be cell-based or may use a cell-free system that recreates or retains the relevant biochemical reaction of the target protein (reviewed in Sittampalam GS *et al.*, Curr Opin Chem Biol (1997) 1:384-91 and accompanying references). As used herein the term "cell-based" refers to assays using live cells, dead cells, or a particular cellular fraction, such as a membrane, endoplasmic reticulum, or mitochondrial fraction. The term "cell free" encompasses assays using substantially purified protein (either endogenous or recombinantly produced), partially purified or crude cellular extracts. Screening assays may detect a variety of molecular events, including

protein-DNA interactions, protein-protein interactions (*e.g.*, receptor-ligand binding), transcriptional activity (*e.g.*, using a reporter gene), enzymatic activity (*e.g.*, via a property of the substrate), activity of second messengers, immunogenicty and changes in cellular morphology or other cellular characteristics. Appropriate screening assays may use a wide range of detection methods including fluorescent, radioactive, colorimetric, spectrophotometric, and amperometric methods, to provide a read-out for the particular molecular event detected.

Cell-based screening assays usually require systems for recombinant expression of IG and any auxiliary proteins demanded by the particular assay. Appropriate methods for generating recombinant proteins produce sufficient quantities of proteins that retain their relevant biological activities and are of sufficient purity to optimize activity and assure assay reproducibility. Yeast two-hybrid and variant screens, and mass spectrometry provide preferred methods for determining protein-protein interactions and elucidation of protein complexes. In certain applications, when IG-interacting proteins are used in screens to identify small molecule modulators, the binding specificity of the interacting protein to the IG protein may be assayed by various known methods such as substrate processing (e.g. ability of the candidate IG-specific binding agents to function as negative effectors in IG-expressing cells), binding equilibrium constants (usually at least about 10^7 M^{-1}, preferably at least about 10^8 M^{-1}, more preferably at least about 10^9 M^{-1}), and immunogenicity (e.g. ability to elicit IG specific antibody in a heterologous host such as a mouse, rat, goat or rabbit). For enzymes and receptors, binding may be assayed by, respectively, substrate and ligand processing.

The screening assay may measure a candidate agent's ability to specifically bind to or modulate activity of an IG polypeptide, a fusion protein thereof, or to cells or membranes bearing the polypeptide or fusion protein. The IG polypeptide can be full length or a fragment thereof that retains functional IG activity. The IG polypeptide may be fused to another polypeptide, such as a peptide tag for detection or anchoring, or to another tag. The IG polypeptide is preferably human IG, or is an ortholog or derivative thereof as described above. In a preferred embodiment, the screening assay detects candidate agent-based modulation of IG interaction with a binding target, such as an endogenous or exogenous protein or other substrate that has IG –specific binding activity, and can be used to assess normal IG gene function.

Suitable assay formats that may be adapted to screen for IG modulators are known in the art. Preferred screening assays are high throughput or ultra high throughput and thus

provide automated, cost-effective means of screening compound libraries for lead compounds (Fernandes PB, Curr Opin Chem Biol (1998) 2:597-603; Sundberg SA, Curr Opin Biotechnol 2000, 11:47-53). In one preferred embodiment, screening assays uses fluorescence technologies, including fluorescence polarization, time-resolved fluorescence, and fluorescence resonance energy transfer. These systems offer means to monitor protein-protein or DNA-protein interactions in which the intensity of the signal emitted from dye-labeled molecules depends upon their interactions with partner molecules (*e.g.*, Selvin PR, Nat Struct Biol (2000) 7:730-4; Fernandes PB, *supra*; Hertzberg RP and Pope AJ, Curr Opin Chem Biol (2000) 4:445-451).

A variety of suitable assay systems may be used to identify candidate IG and p53 pathway modulators (e.g. U.S. Pat. Nos. 5,550,019 and 6,133,437 (apoptosis assays); U.S. Pat. No. 6,020,135 (p53 modulation), among others). Specific preferred assays are described in more detail below.

Apoptosis assays. Assays for apoptosis may be performed by terminal deoxynucleotidyl transferase-mediated digoxigenin-11-dUTP nick end labeling (TUNEL) assay. The TUNEL assay is used to measure nuclear DNA fragmentation characteristic of apoptosis (Lazebnik *et al.*, 1994, Nature 371, 346), by following the incorporation of fluorescein-dUTP (Yonehara *et al.*, 1989, J. Exp. Med. 169, 1747). Apoptosis may further be assayed by acridine orange staining of tissue culture cells (Lucas, R., et al., 1998, Blood 15:4730-41). An apoptosis assay system may comprise a cell that expresses an IG, and that optionally has defective p53 function (e.g. p53 is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the apoptosis assay system and changes in induction of apoptosis relative to controls where no test agent is added, identify candidate p53 modulating agents. In some embodiments of the invention, an apoptosis assay may be used as a secondary assay to test a candidate p53 modulating agents that is initially identified using a cell-free assay system. An apoptosis assay may also be used to test whether IG function plays a direct role in apoptosis. For example, an apoptosis assay may be performed on cells that over- or under-express IG relative to wild type cells. Differences in apoptotic response compared to wild type cells suggests that the IG plays a direct role in the apoptotic response. Apoptosis assays are described further in US Pat. No. 6,133,437.

Cell proliferation and cell cycle assays. Cell proliferation may be assayed via bromodeoxyuridine (BRDU) incorporation. This assay identifies a cell population undergoing DNA synthesis by incorporation of BRDU into newly-synthesized DNA. Newly-synthesized DNA may then be detected using an anti-BRDU antibody (Hoshino *et al.*, 1986, Int. J. Cancer 38, 369; Campana *et al.*, 1988, J. Immunol. Meth. 107, 79), or by other means.

Cell Proliferation may also be examined using [^{3}H]-thymidine incorporation (Chen, J., 1996, Oncogene 13:1395-403; Jeoung, J., 1995, J. Biol. Chem. 270:18367-73). This assay allows for quantitative characterization of S-phase DNA syntheses. In this assay, cells synthesizing DNA will incorporate [^{3}H]-thymidine into newly synthesized DNA. Incorporation can then be measured by standard techniques such as by counting of radioisotope in a scintillation counter (e.g., Beckman LS 3800 Liquid Scintillation Counter).

Cell proliferation may also be assayed by colony formation in soft agar (Sambrook et al., Molecular Cloning, Cold Spring Harbor (1989)). For example, cells transformed with IG are seeded in soft agar plates, and colonies are measured and counted after two weeks incubation.

Involvement of a gene in the cell cycle may be assayed by flow cytometry (Gray JW et al. (1986) Int J Radiat Biol Relat Stud Phys Chem Med 49:237-55). Cells transfected with an IG may be stained with propidium iodide and evaluated in a flow cytometer (available from Becton Dickinson).

Accordingly, a cell proliferation or cell cycle assay system may comprise a cell that expresses an IG, and that optionally has defective p53 function (e.g. p53 is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the assay system and changes in cell proliferation or cell cycle relative to controls where no test agent is added, identify candidate p53 modulating agents. In some embodiments of the invention, the cell proliferation or cell cycle assay may be used as a secondary assay to test a candidate p53 modulating agents that is initially identified using another assay system such as a cell-free assay system. A cell proliferation assay may also be used to test whether IG function plays a direct role in cell proliferation or cell cycle. For example, a cell proliferation or cell cycle assay may be performed on cells that over- or under-express IG relative to wild type cells. Differences in proliferation or cell cycle compared to wild type cells suggests that the IG plays a direct role in cell proliferation or cell cycle.

Angiogenesis. Angiogenesis may be assayed using various human endothelial cell systems, such as umbilical vein, coronary artery, or dermal cells. Suitable assays include Alamar Blue based assays (available from Biosource International) to measure proliferation; migration assays using fluorescent molecules, such as the use of Becton Dickinson Falcon HTS FluoroBlock cell culture inserts to measure migration of cells through membranes in presence or absence of angiogenesis enhancer or suppressors; and tubule formation assays based on the formation of tubular structures by endothelial cells on Matrigel® (Becton Dickinson). Accordingly, an angiogenesis assay system may comprise a cell that expresses an IG, and that optionally has defective p53 function (e.g. p53 is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the angiogenesis assay system and changes in angiogenesis relative to controls where no test agent is added, identify candidate p53 modulating agents. In some embodiments of the invention, the angiogenesis assay may be used as a secondary assay to test a candidate p53 modulating agents that is initially identified using another assay system. An angiogenesis assay may also be used to test whether IG function plays a direct role in cell proliferation. For example, an angiogenesis assay may be performed on cells that over- or under-express IG relative to wild type cells. Differences in angiogenesis compared to wild type cells suggests that the IG plays a direct role in angiogenesis.

Hypoxic induction. The alpha subunit of the transcription factor, hypoxia inducible factor-1 (HIF-1), is upregulated in tumor cells following exposure to hypoxia in vitro. Under hypoxic conditions, HIF-1 stimulates the expression of genes known to be important in tumour cell survival, such as those encoding glyolytic enzymes and VEGF. Induction of such genes by hypoxic conditions may be assayed by growing cells transfected with IG in hypoxic conditions (such as with 0.1% O2, 5% CO2, and balance N2, generated in a Napco 7001 incubator (Precision Scientific)) and normoxic conditions, followed by assessment of gene activity or expression by Taqman®. For example, a hypoxic induction assay system may comprise a cell that expresses an IG, and that optionally has a mutated p53 (e.g. p53 is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the hypoxic induction assay system and changes in hypoxic response relative to controls where no test agent is added, identify candidate p53 modulating agents. In some embodiments of the invention, the hypoxic induction assay may be used as a secondary assay to test a candidate p53 modulating agents that is initially identified using another assay system. A hypoxic induction assay

may also be used to test whether IG function plays a direct role in the hypoxic response. For example, a hypoxic induction assay may be performed on cells that over- or under-express IG relative to wild type cells. Differences in hypoxic response compared to wild type cells suggests that the IG plays a direct role in hypoxic induction.

Cell adhesion. Cell adhesion assays measure adhesion of cells to purified adhesion proteins, or adhesion of cells to each other, in presence or absence of candidate modulating agents. Cell-protein adhesion assays measure the ability of agents to modulate the adhesion of cells to purified proteins. For example, recombinant proteins are produced, diluted to 2.5g/mL in PBS, and used to coat the wells of a microtiter plate. The wells used for negative control are not coated. Coated wells are then washed, blocked with 1% BSA, and washed again. Compounds are diluted to 2× final test concentration and added to the blocked, coated wells. Cells are then added to the wells, and the unbound cells are washed off. Retained cells are labeled directly on the plate by adding a membrane-permeable fluorescent dye, such as calcein-AM, and the signal is quantified in a fluorescent microplate reader.

Cell-cell adhesion assays measure the ability of agents to modulate binding of cell adhesion proteins with their native ligands. These assays use cells that naturally or recombinantly express the adhesion protein of choice. In an exemplary assay, cells expressing the cell adhesion protein are plated in wells of a multiwell plate. Cells expressing the ligand are labeled with a membrane-permeable fluorescent dye, such as BCECF , and allowed to adhere to the monolayers in the presence of candidate agents. Unbound cells are washed off, and bound cells are detected using a fluorescence plate reader.

High-throughput cell adhesion assays have also been described. In one such assay, small molecule ligands and peptides are bound to the surface of microscope slides using a microarray spotter, intact cells are then contacted with the slides, and unbound cells are washed off. In this assay, not only the binding specificity of the peptides and modulators against cell lines are determined, but also the functional cell signaling of attached cells using immunofluorescence techniques in situ on the microchip is measured (Falsey JR et al., Bioconjug Chem. 2001 May-Jun;12(3):346-53).

Primary assays for antibody modulators

For antibody modulators, appropriate primary assays test is a binding assay that tests the antibody's affinity to and specificity for the IG protein. Methods for testing antibody affinity and specificity are well known in the art (Harlow and Lane, 1988, 1999, *supra*). The enzyme-linked immunosorbant assay (ELISA) is a preferred method for detecting IG-specific antibodies; others include FACS assays, radioimmunoassays, and fluorescent assays.

Primary assays for nucleic acid modulators

For nucleic acid modulators, primary assays may test the ability of the nucleic acid modulator to inhibit or enhance IG gene expression, preferably mRNA expression. In general, expression analysis comprises comparing IG expression in like populations of cells (*e.g.*, two pools of cells that endogenously or recombinantly express IG) in the presence and absence of the nucleic acid modulator. Methods for analyzing mRNA and protein expression are well known in the art. For instance, Northern blotting, slot blotting, ribonuclease protection, quantitative RT-PCR (*e.g.*, using the TaqMan®, PE Applied Biosystems), or microarray analysis may be used to confirm that IG mRNA expression is reduced in cells treated with the nucleic acid modulator (*e.g.*, Current Protocols in Molecular Biology (1994) Ausubel FM *et al.*, *eds.*, John Wiley & Sons, Inc., chapter 4; Freeman WM *et al.*, Biotechniques (1999) 26:112-125; Kallioniemi OP, Ann Med 2001, 33:142-147; Blohm DH and Guiseppi-Elie, A Curr Opin Biotechnol 2001, 12:41-47). Protein expression may also be monitored. Proteins are most commonly detected with specific antibodies or antisera directed against either the IG protein or specific peptides. A variety of means including Western blotting, ELISA, or in situ detection, are available (Harlow E and Lane D, 1988 and 1999, *supra*).

Secondary Assays

Secondary assays may be used to further assess the activity of IG-modulating agent identified by any of the above methods to confirm that the modulating agent affects IG in a manner relevant to the p53 pathway. As used herein, IG-modulating agents encompass candidate clinical compounds or other agents derived from previously identified modulating agent. Secondary assays can also be used to test the activity of a modulating agent on a particular genetic or biochemical pathway or to test the specificity of the modulating agent's interaction with IG.

Secondary assays generally compare like populations of cells or animals (*e.g.*, two pools of cells or animals that endogenously or recombinantly express IG) in the presence and absence of the candidate modulator. In general, such assays test whether treatment of cells or animals with a candidate IG–modulating agent results in changes in the p53 pathway in comparison to untreated (or mock- or placebo-treated) cells or animals. Certain assays use "sensitized genetic backgrounds", which, as used herein, describe cells or animals engineered for altered expression of genes in the p53 or interacting pathways.

Cell-based assays

Cell based assays may use a variety of mammalian cell lines known to have defective p53 function (e.g. SAOS-2 osteoblasts, H1299 lung cancer cells, C33A and HT3 cervical cancer cells, HT-29 and DLD-1 colon cancer cells, among others, available from American Type Culture Collection (ATCC), Manassas, VA). Cell based assays may detect endogenous p53 pathway activity or may rely on recombinant expression of p53 pathway components. Any of the aforementioned assays may be used in this cell-based format. Candidate modulators are typically added to the cell media but may also be injected into cells or delivered by any other efficacious means.

Animal Assays

A variety of non-human animal models of normal or defective p53 pathway may be used to test candidate IG modulators. Models for defective p53 pathway typically use genetically modified animals that have been engineered to mis-express (*e.g.*, over-express or lack expression in) genes involved in the p53 pathway. Assays generally require systemic delivery of the candidate modulators, such as by oral administration, injection, etc.

In a preferred embodiment, p53 pathway activity is assessed by monitoring neovascularization and angiogenesis. Animal models with defective and normal p53 are used to test the candidate modulator's affect on IG in Matrigel® assays. Matrigel® is an extract of basement membrane proteins, and is composed primarily of laminin, collagen IV, and heparin sulfate proteoglycan. It is provided as a sterile liquid at 4° C, but rapidly forms a solid gel at 37° C. Liquid Matrigel® is mixed with various angiogenic agents, such as bFGF and VEGF, or with human tumor cells which over-express the IG. The mixture is then injected subcutaneously(SC) into female athymic nude mice (Taconic, Germantown, NY) to support an intense vascular response. Mice with Matrigel® pellets

may be dosed via oral (PO), intraperitoneal (IP), or intravenous (IV) routes with the candidate modulator. Mice are euthanized 5 - 12 days post-injection, and the Matrigel® pellet is harvested for hemoglobin analysis (Sigma plasma hemoglobin kit). Hemoglobin content of the gel is found to correlate the degree of neovascularization in the gel.

In another preferred embodiment, the effect of the candidate modulator on IG is assessed via tumorigenicity assays. In one example, xenograft human tumors are implanted SC into female athymic mice, 6-7 week old, as single cell suspensions either from a pre-existing tumor or from *in vitro* culture. The tumors which express the IG endogenously are injected in the flank, 1 x 10^5 to 1 x 10^7 cells per mouse in a volume of 100 μL using a 27gauge needle. Mice are then ear tagged and tumors are measured twice weekly. Candidate modulator treatment is initiated on the day the mean tumor weight reaches 100 mg. Candidate modulator is delivered IV, SC, IP, or PO by bolus administration. Depending upon the pharmacokinetics of each unique candidate modulator, dosing can be performed multiple times per day. The tumor weight is assessed by measuring perpendicular diameters with a caliper and calculated by multiplying the measurements of diameters in two dimensions. At the end of the experiment, the excised tumors maybe utilized for biomarker identification or further analyses. For immunohistochemistry staining, xenograft tumors are fixed in 4% paraformaldehyde, 0.1M phosphate, pH 7.2, for 6 hours at 4°C, immersed in 30% sucrose in PBS, and rapidly frozen in isopentane cooled with liquid nitrogen.

Diagnostic and therapeutic uses

Specific IG-modulating agents are useful in a variety of diagnostic and therapeutic applications where disease or disease prognosis is related to defects in the p53 pathway, such as angiogenic, apoptotic, or cell proliferation disorders. Accordingly, the invention also provides methods for modulating the p53 pathway in a cell, preferably a cell pre-determined to have defective p53 function, comprising the step of administering an agent to the cell that specifically modulates IG activity. Preferably, the modulating agent produces a detectable phenotypic change in the cell indicating that the p53 function is restored, i.e., for example, the cell undergoes normal proliferation or progression through the cell cycle.

The discovery that IG is implicated in p53 pathway provides for a variety of methods that can be employed for the diagnostic and prognostic evaluation of diseases and

disorders involving defects in the p53 pathway and for the identification of subjects having a predisposition to such diseases and disorders.

Various expression analysis methods can be used to diagnose whether IG expression occurs in a particular sample, including Northern blotting, slot blotting, ribonuclease protection, quantitative RT-PCR, and microarray analysis. (*e.g.*, Current Protocols in Molecular Biology (1994) Ausubel FM *et al.*, *eds.*, John Wiley & Sons, Inc., chapter 4; Freeman WM *et al.*, Biotechniques (1999) 26:112-125; Kallioniemi OP, Ann Med 2001, 33:142-147; Blohm and Guiseppi-Elie, Curr Opin Biotechnol 2001, 12:41-47). Tissues having a disease or disorder implicating defective p53 signaling that express an IG, are identified as amenable to treatment with an IG modulating agent. In a preferred application, the p53 defective tissue overexpresses an IG relative to normal tissue. For example, a Northern blot analysis of mRNA from tumor and normal cell lines, or from tumor and matching normal tissue samples from the same patient, using full or partial IG cDNA sequences as probes, can determine whether particular tumors express or overexpress IG. Alternatively, the TaqMan® is used for quantitative RT-PCR analysis of IG expression in cell lines, normal tissues and tumor samples (PE Applied Biosystems).

Various other diagnostic methods may be performed, for example, utilizing reagents such as the IG oligonucleotides, and antibodies directed against an IG, as described above for: (1) the detection of the presence of IG gene mutations, or the detection of either over- or under-expression of IG mRNA relative to the non-disorder state; (2) the detection of either an over- or an under-abundance of IG gene product relative to the non-disorder state; and (3) the detection of perturbations or abnormalities in the signal transduction pathway mediated by IG.

Thus, in a specific embodiment, the invention is drawn to a method for diagnosing a disease in a patient, the method comprising: a) obtaining a biological sample from the patient; b) contacting the sample with a probe for IG expression; c) comparing results from step (b) with a control; and d) determining whether step (c) indicates a likelihood of disease. Preferably, the disease is cancer, most preferably a cancer as shown in TABLE 2. The probe may be either DNA or protein, including an antibody.

EXAMPLES

The following experimental section and examples are offered by way of illustration and not by way of limitation.

I. Drosophila p53 screen

The Drosophila p53 gene was overexpressed specifically in the wing using the vestigial margin quadrant enhancer. Increasing quantities of Drosophila p53 (titrated using different strength transgenic inserts in 1 or 2 copies) caused deterioration of normal wing morphology from mild to strong, with phenotypes including disruption of pattern and polarity of wing hairs, shortening and thickening of wing veins, progressive crumpling of the wing and appearance of dark "death" inclusions in wing blade. In a screen designed to identify enhancers and suppressors of Drosophila p53, homozygous females carrying two copies of p53 were crossed to 5663 males carrying random insertions of a piggyBac transposon (Fraser M *et al.*, Virology (1985) 145:356-361). Progeny containing insertions were compared to non-insertion-bearing sibling progeny for enhancement or suppression of the p53 phenotypes. Sequence information surrounding the piggyBac insertion site was used to identify the modifier genes. Modifiers of the wing phenotype were identified as members of the p53 pathway. CG14372 was an enhancer of the wing phenotype. Human orthologs of the modifiers are referred to herein as IG.

BLAST analysis (Altschul et al., *supra*) was employed to identify Targets from Drosophila modifiers. For example, representative sequences from IG, GI#s 12310959, 3451335, 7705413, 16182764, 5918159, and 11067409, (SEQ ID NOs: 44, 46, 50, 53, 49, 57, respectively), share 22%, 26%, 33%, 23%, 31%, and 29% amino acid identity, respectively, with the *Drosophila* CG14372.

Various domains, signals, and functional subunits in proteins were analyzed using the PSORT (Nakai K., and Horton P., Trends Biochem Sci, 1999, 24:34-6; Kenta Nakai, Protein sorting signals and prediction of subcellular localization, Adv. Protein Chem. 54, 277-344 (2000)), PFAM (Bateman A., et al., Nucleic Acids Res, 1999, 27:260-2; http://pfam.wustl.edu), SMART (Ponting CP, et al., SMART: identification and annotation of domains from signaling and extracellular protein sequences. Nucleic Acids Res. 1999 Jan 1;27(1):229-32), TM-HMM (Erik L.L. Sonnhammer, Gunnar von Heijne, and Anders Krogh: A hidden Markov model for predicting transmembrane helices in protein sequences. In Proc. of Sixth Int. Conf. on Intelligent Systems for Molecular Biology, p 175-182 Ed J. Glasgow, T. Littlejohn, F. Major, R. Lathrop, D. Sankoff, and C. Sensen Menlo Park, CA: AAAI Press, 1998), and clust (Remm M, and Sonnhammer E. Classification of transmembrane protein families in the Caenorhabditis elegans genome and identification of human orthologs. Genome Res. 2000 Nov;10(11):1679-89) programs.

Representative immunoglobulin, immunoglobulin-like, and transmembrane domains of various IGs are outlined in Table 1.

Table 1

Target GI#	SEQ ID NO	Immunoglobulin domain (PFAM00047)	Immunoglobulin-like domain (SMART SM0410)	Transmembrane domain start/end (TM-HMM)
12310959	44	46 to 115, 148 to 214, 250 to 307	38 to 130, 242 to 323	(336,358)
11386199	45	71 to 150, 186 to 248, 284 to 340	63 to 167	(404,426)
3451335	46	15 to 84, 116 to 179, 216 to 271	7 to 99, 108 to 199, 208 to 287	(317,339)
7705413	50	50 to 117, 150 to 203, 236 to 297	42 to 133, 142 to 220, 228 to 320	No TMs
16182764	53	63 to 130, 164 to 230, 265 to 317, 350 to 401, 434 to 502	55 to 145, 156 to 248, 342 to 418, 426 to 518	(537,559)
16716339	55	45 to 129, 162 to 225, 263 to 317	37 to 146, 257 to 333	(350,372)
5918159	49	77 to 146, 179 to 245, 281 to 335	69 to 161, 171 to 264, 273 to 351	(364,386)
11067409	57	47 to 114, 147 to 199, 232 to 293	39 to 130, 139 to 216, 224 to 309	No TMs
4505025	58	46 to 113, 146 to 199, 232 to 292	38 to 129, 138 to 216, 224 to 308	(313,335)
4505505	59	50 to 104, 137 to 198	42 to 121, 129 to 214	No TMs
11602906	60	41 to 123, 259 to 315	33 to 140, 154 to 241, 251 to 331	(353,375)
5360210	62	47 to 142, 176 to 240, 276 to 331	39 to 159, 270 to 347	(361,383)
8394411	63	48 to 132, 266 to 323	40 to 149, 260 to 339	(353,375)
	47	29 to 121, 130 to 221, 230 to 309	29 to 121, 130 to 221, 230 to 309	(332,352)

II. High-Throughput In Vitro Fluorescence Polarization Assay

Fluorescently-labeled IG peptide/substrate are added to each well of a 96-well microtiter plate, along with a test agent in a test buffer (10 mM HEPES, 10 mM NaCl, 6 mM magnesium chloride, pH 7.6). Changes in fluorescence polarization, determined by using a Fluorolite FPM-2 Fluorescence Polarization Microtiter System (Dynatech Laboratories, Inc), relative to control values indicates the test compound is a candidate modifier of IG activity.

III. High-Throughput In Vitro Binding Assay.

^{33}P-labeled IG peptide is added in an assay buffer (100 mM KCl, 20 mM HEPES pH 7.6, 1 mM $MgCl_2$, 1% glycerol, 0.5% NP-40, 50 mM beta-mercaptoethanol, 1 mg/ml BSA, cocktail of protease inhibitors) along with a test agent to the wells of a Neutralite-avidin coated assay plate and incubated at 25°C for 1 hour. Biotinylated substrate is then added to each well and incubated for 1 hour. Reactions are stopped by washing with PBS, and counted in a scintillation counter. Test agents that cause a difference in activity relative to control without test agent are identified as candidate p53 modulating agents.

IV. Immunoprecipitations and Immunoblotting

For coprecipitation of transfected proteins, 3×10^6 appropriate recombinant cells containing the IG proteins are plated on 10-cm dishes and transfected on the following day with expression constructs. The total amount of DNA is kept constant in each transfection by adding empty vector. After 24 h, cells are collected, washed once with phosphate-buffered saline and lysed for 20 min on ice in 1 ml of lysis buffer containing 50 mM Hepes, pH 7.9, 250 mM NaCl, 20 mM -glycerophosphate, 1 mM sodium orthovanadate, 5 mM p-nitrophenyl phosphate, 2 mM dithiothreitol, protease inhibitors (complete, Roche Molecular Biochemicals), and 1% Nonidet P-40. Cellular debris is removed by centrifugation twice at 15,000 × g for 15 min. The cell lysate is incubated with 25 μl of M2 beads (Sigma) for 2 h at 4 °C with gentle rocking.

After extensive washing with lysis buffer, proteins bound to the beads are solubilized by boiling in SDS sample buffer, fractionated by SDS-polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membrane and blotted with the indicated antibodies. The reactive bands are visualized with horseradish peroxidase coupled to the appropriate secondary antibodies and the enhanced chemiluminescence (ECL) Western blotting detection system (Amersham Pharmacia Biotech).

V. Expression analysis

All cell lines used in the following experiments are NCI (National Cancer Institute) lines, and are available from ATCC (American Type Culture Collection, Manassas, VA 20110-2209). Normal and tumor tissues were obtained from Impath, UC Davis, Clontech, Stratagene, and Ambion.

TaqMan analysis was used to assess expression levels of the disclosed genes in various samples.

RNA was extracted from each tissue sample using Qiagen (Valencia, CA) RNeasy kits, following manufacturer's protocols, to a final concentration of 50ng/μl. Single stranded cDNA was then synthesized by reverse transcribing the RNA samples using random hexamers and 500ng of total RNA per reaction, following protocol 4304965 of Applied Biosystems (Foster City, CA, http://www.appliedbiosystems.com/).

Primers for expression analysis using TaqMan assay (Applied Biosystems, Foster City, CA) were prepared according to the TaqMan protocols, and the following criteria: a) primer pairs were designed to span introns to eliminate genomic contamination, and b) each primer pair produced only one product.

Taqman reactions were carried out following manufacturer's protocols, in 25 μl total volume for 96-well plates and 10 μl total volume for 384-well plates, using 300nM primer and 250 nM probe, and approximately 25ng of cDNA. The standard curve for result analysis was prepared using a universal pool of human cDNA samples, which is a mixture of cDNAs from a wide variety of tissues so that the chance that a target will be present in appreciable amounts is good. The raw data were normalized using 18S rRNA (universally expressed in all tissues and cells).

For each expression analysis, tumor tissue samples were compared with matched normal tissues from the same patient. A gene was considered overexpressed in a tumor when the level of expression of the gene was 2 fold or higher in the tumor compared with its matched normal sample. In cases where normal tissue was not available, a universal pool of cDNA samples was used instead. In these cases, a gene was considered overexpressed in a tumor sample when the difference of expression levels between a tumor sample and the average of all normal samples from the same tissue type was greater than 2 times the standard deviation of all normal samples (i.e., Tumor – average(all normal samples) > 2 x STDEV(all normal samples)).

Results are shown in Table 2. Data presented in bold indicate that greater than 50% of tested tumor samples of the tissue type indicated in row 1 exhibited over expression of the

gene listed in column 1, relative to normal samples. Underlined data indicates that between 25% to 49% of tested tumor samples exhibited over expression. A modulator identified by an assay described herein can be further validated for therapeutic effect by administration to a tumor in which the gene is overexpressed. A decrease in tumor growth confirms therapeutic utility of the modulator. Prior to treating a patient with the modulator, the likelihood that the patient will respond to treatment can be diagnosed by obtaining a tumor sample from the patient, and assaying for expression of the gene targeted by the modulator. The expression data for the gene(s) can also be used as a diagnostic marker for disease progression. The assay can be performed by expression analysis as described above, by antibody directed to the gene target, or by any other available detection method.

Table 2

	breast			colon			kidney			lung			ovary	
GI#12310958 (SEQ ID NO:1)	1	3		1	26		**11**	**19**		0	14		0	4
GI#11386198 (SEQ ID NO:4)	0	12		4	30		0	0		1	14		3	7
GI#3451333 (SEQ ID NO:6)	0	3		7	26		2	19		**7**	**14**		**2**	4
GI#7705412 (SEQ ID NO:16)	1	3		11	26		2	19		2	14		0	4
GI#9049507 (SEQ ID NO:23)	1	3		15	26		2	19		**9**	**14**		**3**	**4**
GI#5457320 (SEQ ID NO:11)	0	3		3	26		**14**	**19**		1	14		0	4
GI#4505024 (SEQ ID NO:28)	4	12		6	30		0	0		0	14		0	7
GI#4505504 (SEQ ID NO:35)	1	12		10	26		0	0		5	13		0	5
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VI. Full length cloning of F22162

The genomic fragment GI#15789228 (SEQ ID NO:8) was identified as the human F22162 target sequence. The cDNA sequence corresponding to the translation product for the F22162, GI#3451335 (SEQ ID NO:46) was blasted at the nucleotide level against all the available EST databases. This approach identified a number of ESTs that span the entire length of the F22162 ref seq and extended the sequence towards the N-terminus (5' end). When all of the ESTs were assembled, an extended open reading frame was obtained containing a good Kozak consensus sequence for translational initiation. Based

on the predicted cDNA sequence, several PCR primers were synthesized and used for amplification reactions to obtain the full-length sequences. The mRNA from 26 different normal tissues and total RNA from 8 tumor sources purchased from Clontech was used to generate single stranded cDNA in a Reverse Transcription reaction. The pooled single-stranded cDNA was used as template for PCR amplification reactions. PCR products were subcloned into the vector pCRII-topo from Invitrogen. Colonies were picked and the inserted DNA was sequenced both directions. Two forms of the gene were identified: a long form (SEQ ID NO:9), and a short form (SEQ ID NO:10). The transmembrane domain in missing in the short form, and thus this variant may be soluble.

WHAT IS CLAIMED IS:

1. A method of identifying a candidate p53 pathway modulating agent, said method comprising the steps of:

(a) providing an assay system comprising a purified IG polypeptide or nucleic acid or a functionally active fragment or derivative thereof;

(b) contacting the assay system with a test agent under conditions whereby, but for the presence of the test agent, the system provides a reference activity; and

(c) detecting a test agent-biased activity of the assay system, wherein a difference between the test agent-biased activity and the reference activity identifies the test agent as a candidate p53 pathway modulating agent.

2. The method of Claim 1 wherein the assay system comprises cultured cells that express the IG polypeptide.

3. The method of Claim 2 wherein the cultured cells additionally have defective p53 function.

4. The method of Claim 1 wherein the assay system includes a screening assay comprising an IG polypeptide, and the candidate test agent is a small molecule modulator.

5. The method of Claim 4 wherein the assay is a binding assay.

6. The method of Claim 1 wherein the assay system is selected from the group consisting of an apoptosis assay system, a cell proliferation assay system, an angiogenesis assay system, and a hypoxic induction assay system.

7. The method of Claim 1 wherein the assay system includes a binding assay comprising an IG polypeptide and the candidate test agent is an antibody.

8. The method of Claim 1 wherein the assay system includes an expression assay comprising an IG nucleic acid and the candidate test agent is a nucleic acid modulator.

9. The method of claim 8 wherein the nucleic acid modulator is an antisense oligomer.

10. The method of Claim 8 wherein the nucleic acid modulator is a PMO.

11. The method of Claim 1 additionally comprising:

(d) administering the candidate p53 pathway modulating agent identified in (c) to a model system comprising cells defective in p53 function and, detecting a phenotypic change in the model system that indicates that the p53 function is restored.

12. The method of Claim 11 wherein the model system is a mouse model with defective p53 function.

13. A method for modulating a p53 pathway of a cell comprising contacting a cell defective in p53 function with a candidate modulator that specifically binds to an IG polypeptide comprising an amino acid sequence selected from group consisting of SEQ ID NOs:44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, and 63, whereby p53 function is restored.

14. The method of claim 13 wherein the candidate modulator is administered to a vertebrate animal predetermined to have a disease or disorder resulting from a defect in p53 function.

15. The method of Claim 13 wherein the candidate modulator is selected from the group consisting of an antibody and a small molecule.

16. The method of Claim 1, comprising the additional steps of:

(d) providing a secondary assay system comprising cultured cells or a non-human animal expressing IG ,

(e) contacting the secondary assay system with the test agent of (b) or an agent derived therefrom under conditions whereby, but for the presence of the test agent or agent derived therefrom, the system provides a reference activity; and

(f) detecting an agent-biased activity of the second assay system,

wherein a difference between the agent-biased activity and the reference activity of the second assay system confirms the test agent or agent derived therefrom as a candidate p53 pathway modulating agent,
and wherein the second assay detects an agent-biased change in the p53 pathway.

17. The method of Claim 16 wherein the secondary assay system comprises cultured cells.

18. The method of Claim 16 wherein the secondary assay system comprises a non-human animal.

19. The method of Claim 18 wherein the non-human animal mis-expresses a p53 pathway gene.

20. A method of modulating p53 pathway in a mammalian cell comprising contacting the cell with an agent that specifically binds an IG polypeptide or nucleic acid.

21. The method of Claim 20 wherein the agent is administered to a mammalian animal predetermined to have a pathology associated with the p53 pathway.

22. The method of Claim 20 wherein the agent is a small molecule modulator, a nucleic acid modulator, or an antibody.

23. A method for diagnosing a disease in a patient comprising:
(a) obtaining a biological sample from the patient;
(b) contacting the sample with a probe for IG expression;
(c) comparing results from step (b) with a control;
(d) determining whether step (c) indicates a likelihood of disease.

24. The method of claim 23 wherein said disease is cancer.

25. The method according to claim 24, wherein said cancer is a cancer as shown in Table 2 as having >25% expression level.

26. A purified nucleic acid molecule that encodes a polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:47, or reverse complement thereof.

27. The nucleic acid molecule of Claim 26 which is capable of hybridizing to a nucleic acid sequence of SEQ ID NO:9 using high stringency hybridization conditions.

28. A recombinant expression system comprising a DNA or RNA molecule, wherein said expression system is capable of producing an IG polypeptide comprising the amino acid sequence of SEQ ID NO:47 when said expression system is present in a compatible host cell.

29. A host cell comprising the expression system of claim 28.

30. A process for producing an IG protein comprising culturing the host cell of Claim 29 under conditions suitable for expression of said IG protein and recovering said protein.

31. A process for producing a cell which produces an IG protein comprising the transformation or transfection of a host cell with the expression system of claim 28 such that the host cell, under appropriate culture conditions, produces an IG protein.

32. A recombinant host cell expressing the protein produced by the method of claim 31.

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agctgccgtg ccacctgctg ccacctgttc ctggactgta catctccctg gtgacctggc 360

agcgcccaga tgcacctgcg aaccaccaga atgtggccgc cttccaccct aagatgggtc 420

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gcactgggca agacacagag gcagagctcc aggacgccac gctggccctc cacgggctca 540

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tagtggggat ccagagagga ccccccgccc cagagacttg gttttggctc cagccttccc 1860

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aaaaaaaa 1928

<210> 42
<211> 2442
<212> DNA
<213> Homo sapiens

<400> 42
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ctaaaccgcc cagccgatcg gcccccacag agtggcccgc gggcctccgg ccgggcccag 180

tcccctcccg ggccctccat ggcccgggcc gctgccctcc tgccgtcgag atcgccgccg 240

acgccgctgc tgtggccgct gctgctgctg ctgctcctgg aaaccggagc ccaggatgtg 300

cgagttcaag tgctacccga ggtgcgaggc cagctcgggg gcaccgtgga gctgccgtgc 360

cacctgctgc cacctgttcc tggactgtac atctccctgg tgacctggca gcgcccagat 420

gcacctgcga accaccagaa tgtggccgcc ttccacccta agatgggtcc cagcttcccc 480

agcccgaagc ctggcagcga gcggctgtcc ttcgtctctg ccaagcagag cactgggcaa 540

gacacagagg cagagctcca ggacgccacg ctggccctcc acgggctcac ggtggaggac 600

gagggcaact acacttgcga gtttgccacc ttccccaagg ggtccgtccg agggatgacc 660

tggctcagag tcatagccaa gcccaagaac caagctgagg cccagaaggt cacgttcagc 720

caggacccta cgacagtggc cctctgcatc tccaaagagg gccgcccacc tgcccggatc 780

tcctggctct catccctgga ctgggaagcc aaagagactc aggtgtcagg gaccctggcc 840

ggaactgtca ctgtcaccag ccgcttcacc ttggtgccct cgggccgagc agatggtgtc 900

acggtcacct gcaaagtgga gcatgagagc ttcgaggaac cagccctgat acctgtgacc 960

ctctctgtac gctaccctcc tgaagtgtcc atctccggct atgatgacaa ctggtacctc 1020

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gctttcttgc caaggatcta gtgccccctg acctctggcc aggccactgt cagttaacac 1920

atatgcattc catttgtgat gtctaccttg gtggctccac tatgacccct aacccatgag 1980

cccagagaaa ttcaccgtga taatggaatc ctggcaacct tatctcatga ggcaggaggt 2040

ggggaaggtg cttctgcaca acctctgatc ccaaggactc ctctcccaga ctgtgacctt 2100

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acctataatt tgtcttcagg tagtaaattc ctgcctacca agcaagcagc cccagcctag 2220

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tttgtaaact acatttccta caaaatatga atttatactt tg 2442

<210> 43
<211> 2171
<212> DNA
<213> Rattus norvegicus

<400> 43
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tggagccctc tcccaccttg atgccgcagg acgtggccaa atgcatctct gctgatggtc 600

accctcctgg acgaatcacg tggtcctcga atgtgaatgg aagctaccgt gaaatgaagg 660

aaacagggtc ccagccgggc accaccacag ttatcagcta cctctccatg gtgccttcta 720

gccaggcaga tggcacgaac atcacctgca cagtggaaca tgaaagcttc caggagccgg 780

accagcagcc attgatcctt tccctacctt atccacccga agtgtccatc tctggctatg 840

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caccgcccac caactatagc tggagcacgg ccacgggtcc ccttcccaac tccactcatt 960

tccaggaaaa cggcagtcac ctgctaatct ccaccgtgga tgacctcaat aacacgatct 1020

ttgtgtgcaa agccatcaat gccctagggt ctgggcaggg ccaagtgacc atcctagtta 1080

aagaggcatc tgagattctg ccgccaaaga caagcttagg cactggctac atcattgcca 1140

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agaactaagg aacttgaaga cataacaact ggaaccctac ttccacaaaa gaaaaagcct 1380

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tatttttga gacagtgttt cctgtagccc aggctgacct caaacttact atgtagccaa 1980

gaatgatagt aaactaattt attttaattt gtcttcaagc ttaaacatag cccaacccct 2040

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ataaaaaaaa g 2171

<210> 44
<211> 404
<212> PRT
<213> Homo sapiens

<400> 44

Met Ile Trp Lys Arg Ser Ala Val Leu Arg Phe Tyr Ser Val Cys Gly
1 5 10 15

Leu Leu Leu Gln Ala Ala Ala Ser Lys Asn Lys Val Lys Gly Ser Gln
20 25 30

Gly Gln Phe Pro Leu Thr Gln Asn Val Thr Val Val Glu Gly Gly Thr
35 40 45

```
Ala Ile Leu Thr Cys Arg Val Asp Gln Asn Asp Asn Thr Ser Leu Gln
    50                  55                  60

Trp Ser Asn Pro Ala Gln Gln Thr Leu Tyr Phe Asp Asp Lys Lys Ala
65                  70                  75                  80

Leu Arg Asp Asn Arg Ile Glu Leu Val Arg Ala Ser Trp His Glu Leu
                85                  90                  95

Ser Ile Ser Val Ser Asp Val Ser Leu Ser Asp Glu Gly Gln Tyr Thr
            100                 105                 110

Cys Ser Leu Phe Thr Met Pro Val Lys Thr Ser Lys Ala Tyr Leu Thr
        115                 120                 125

Val Leu Gly Val Pro Glu Lys Pro Gln Ile Ser Gly Phe Ser Ser Pro
    130                 135                 140

Val Met Glu Gly Asp Leu Met Gln Leu Thr Cys Lys Thr Ser Gly Ser
145                 150                 155                 160

Lys Pro Ala Ala Asp Ile Arg Trp Phe Lys Asn Asp Lys Glu Ile Lys
                165                 170                 175

Asp Val Lys Tyr Leu Lys Glu Glu Asp Ala Asn Arg Lys Thr Phe Thr
            180                 185                 190

Val Ser Ser Thr Leu Asp Phe Arg Val Asp Arg Ser Asp Asp Gly Val
        195                 200                 205

Ala Val Ile Cys Arg Val Asp His Glu Ser Leu Asn Ala Thr Pro Gln
    210                 215                 220

Val Ala Met Gln Val Leu Glu Ile His Tyr Thr Pro Ser Val Lys Ile
225                 230                 235                 240

Ile Pro Ser Thr Pro Phe Pro Gln Glu Gly Gln Pro Leu Ile Leu Thr
                245                 250                 255

Cys Glu Ser Lys Gly Lys Pro Leu Pro Glu Pro Val Leu Trp Thr Lys
            260                 265                 270
```

Asp Gly Gly Glu Leu Pro Asp Pro Asp Arg Met Val Val Ser Gly Arg
275 280 285

Glu Leu Asn Ile Leu Phe Leu Asn Lys Thr Asp Asn Gly Thr Tyr Arg
290 295 300

Cys Glu Ala Thr Asn Thr Ile Gly Gln Ser Ser Ala Glu Tyr Val Leu
305 310 315 320

Ile Val His Asp Pro Asn Ala Leu Ala Gly Gln Asn Gly Pro Asp His
325 330 335

Ala Leu Ile Gly Gly Ile Val Ala Val Val Val Phe Val Thr Leu Cys
340 345 350

Ser Ile Phe Leu Leu Gly Arg Tyr Leu Ala Arg His Lys Gly Thr Tyr
355 360 365

Leu Thr Asn Glu Ala Lys Gly Ala Glu Asp Ala Pro Asp Ala Asp Thr
370 375 380

Ala Ile Ile Asn Ala Glu Gly Ser Gln Val Asn Ala Glu Glu Lys Lys
385 390 395 400

Glu Tyr Phe Ile

<210> 45
<211> 549
<212> PRT
<213> Homo sapiens

<400> 45

Met Ala Arg Thr Leu Arg Pro Ser Pro Leu Cys Pro Gly Gly Gly Lys
1 5 10 15

Ala Gln Leu Ser Ser Ala Ser Leu Leu Gly Ala Gly Leu Leu Leu Gln
20 25 30

Pro Pro Thr Pro Pro Pro Leu Leu Leu Leu Leu Phe Pro Leu Leu Leu
35 40 45

Phe Ser Arg Leu Cys Gly Ala Leu Ala Gly Pro Ile Ile Val Glu Pro

```
    50                  55                  60

His Val Thr Ala Val Trp Gly Lys Asn Val Ser Leu Lys Cys Leu Ile
65                  70                  75                  80

Glu Val Asn Glu Thr Ile Thr Gln Ile Ser Trp Glu Lys Ile His Gly
                85                  90                  95

Lys Ser Ser Gln Thr Val Ala Val His His Pro Gln Tyr Gly Phe Ser
            100                 105                 110

Val Gln Gly Glu Tyr Gln Gly Arg Val Leu Phe Lys Asn Tyr Ser Leu
        115                 120                 125

Asn Asp Ala Thr Ile Thr Leu His Asn Ile Gly Phe Ser Asp Ser Gly
    130                 135                 140

Lys Tyr Ile Cys Lys Ala Val Thr Phe Pro Leu Gly Asn Ala Gln Ser
145                 150                 155                 160

Ser Thr Thr Val Thr Val Leu Val Glu Pro Thr Val Ser Leu Ile Lys
                165                 170                 175

Gly Pro Asp Ser Leu Ile Asp Gly Gly Asn Glu Thr Val Ala Ala Ile
            180                 185                 190

Cys Ile Ala Ala Thr Gly Lys Pro Val Ala His Ile Asp Trp Glu Gly
        195                 200                 205

Asp Leu Gly Glu Met Glu Ser Thr Thr Thr Ser Phe Pro Asn Glu Thr
    210                 215                 220

Ala Thr Ile Ile Ser Gln Tyr Lys Leu Phe Pro Thr Arg Phe Ala Arg
225                 230                 235                 240

Gly Arg Arg Ile Thr Cys Val Val Lys His Pro Ala Leu Glu Lys Asp
                245                 250                 255

Ile Arg Tyr Ser Phe Ile Leu Asp Ile Gln Tyr Ala Pro Glu Val Ser
            260                 265                 270

Val Thr Gly Tyr Asp Gly Asn Trp Phe Val Gly Arg Lys Gly Val Asn
        275                 280                 285
```

```
Leu Lys Cys Asn Ala Asp Ala Asn Pro Pro Pro Phe Lys Ser Val Trp
    290                 295                 300

Ser Arg Leu Asp Gly Gln Trp Pro Asp Gly Leu Leu Ala Ser Asp Asn
305                 310                 315                 320

Thr Leu His Phe Val His Pro Leu Thr Phe Asn Tyr Ser Gly Val Tyr
                325                 330                 335

Ile Cys Lys Val Thr Asn Ser Leu Gly Gln Arg Ser Asp Gln Lys Val
            340                 345                 350

Ile Tyr Ile Ser Asp Pro Pro Thr Thr Thr Thr Leu Gln Pro Thr Ile
        355                 360                 365

Gln Trp His Pro Ser Thr Ala Asp Ile Glu Asp Leu Ala Thr Glu Pro
    370                 375                 380

Lys Lys Leu Pro Phe Pro Leu Ser Thr Leu Ala Thr Ile Lys Asp Asp
385                 390                 395                 400

Thr Ile Ala Thr Ile Ile Ala Ser Val Val Gly Gly Ala Leu Phe Ile
                405                 410                 415

Val Leu Val Ser Val Leu Ala Gly Ile Phe Cys Tyr Arg Arg Arg Arg
            420                 425                 430

Thr Phe Arg Gly Asp Tyr Phe Ala Lys Asn Tyr Ile Pro Pro Ser Asp
        435                 440                 445

Met Gln Lys Glu Ser Gln Ile Asp Val Leu Gln Gln Asp Glu Leu Asp
    450                 455                 460

Ser Tyr Pro Asp Ser Val Lys Lys Glu Asn Lys Asn Pro Val Asn Asn
465                 470                 475                 480

Leu Ile Arg Lys Asp Tyr Leu Glu Glu Pro Glu Lys Thr Gln Trp Asn
                485                 490                 495

Asn Val Glu Asn Leu Asn Arg Phe Glu Arg Pro Met Asp Tyr Tyr Glu
            500                 505                 510
```

```
Asp Leu Lys Met Gly Met Lys Phe Val Ser Asp Glu His Tyr Asp Glu
        515                 520                 525

Asn Glu Asp Asp Leu Val Ser His Val Asp Gly Ser Val Ile Ser Arg
    530                 535                 540

Arg Glu Trp Tyr Val
545
```

<210> 46
<211> 381
<212> PRT
<213> Homo sapiens

<400> 46

```
Ala Gly Gln Glu Val Gln Thr Glu Asn Val Thr Val Ala Glu Gly Gly
1               5                   10                  15

Val Ala Glu Ile Thr Cys Arg Leu His Gln Tyr Asp Gly Ser Ile Val
            20                  25                  30

Val Ile Gln Asn Pro Ala Arg Gln Thr Leu Phe Phe Asn Gly Thr Arg
        35                  40                  45

Ala Leu Lys Asp Glu Arg Phe Gln Leu Glu Glu Phe Ser Pro Arg Arg
    50                  55                  60

Val Arg Ile Arg Leu Ser Asp Ala Arg Leu Glu Asp Glu Gly Gly Tyr
65                  70                  75                  80

Phe Cys Gln Leu Tyr Thr Glu Asp Thr His His Gln Ile Ala Thr Leu
                85                  90                  95

Thr Val Leu Val Ala Pro Glu Asn Pro Val Val Glu Val Arg Glu Gln
            100                 105                 110

Ala Val Glu Gly Gly Glu Val Glu Leu Ser Cys Leu Val Pro Arg Ser
        115                 120                 125

Arg Pro Ala Ala Thr Leu Arg Trp Tyr Arg Asp Arg Lys Glu Leu Lys
    130                 135                 140

Gly Val Ser Ser Ser Gln Glu Asn Gly Lys Val Trp Ser Val Ala Ser
```

```
145                 150                 155                 160

Thr Val Arg Phe Arg Val Asp Arg Lys Asp Asp Gly Gly Ile Ile Ile
                165                 170                 175

Cys Glu Ala Gln Asn Gln Ala Leu Pro Ser Gly His Ser Lys Gln Thr
            180                 185                 190

Gln Tyr Val Leu Asp Val Gln Tyr Ser Pro Thr Ala Arg Ile His Ala
        195                 200                 205

Ser Gln Ala Val Val Arg Glu Gly Asp Thr Leu Val Leu Thr Cys Ala
    210                 215                 220

Val Thr Gly Asn Pro Arg Pro Asn Gln Ile Arg Trp Asn Arg Gly Asn
225                 230                 235                 240

Glu Ser Leu Pro Glu Arg Ala Glu Ala Val Gly Glu Thr Leu Thr Leu
                245                 250                 255

Pro Gly Leu Val Ser Ala Asp Asn Gly Thr Tyr Thr Cys Glu Ala Ser
            260                 265                 270

Asn Lys His Gly His Ala Arg Ala Leu Tyr Val Leu Val Val Tyr Gly
        275                 280                 285

Glu Ser Arg Leu Arg Pro Thr Glu Gly Gly Gly Gly Ala Pro Asp Pro
    290                 295                 300

Gly Ala Val Val Glu Ala Gln Thr Ser Val Pro Tyr Ala Ile Val Gly
305                 310                 315                 320

Gly Ile Leu Ala Leu Leu Val Phe Leu Ile Ile Cys Val Leu Val Gly
                325                 330                 335

Met Val Trp Cys Ser Val Arg Gln Lys Gly Ser Tyr Leu Thr His Glu
            340                 345                 350

Ala Ser Gly Leu Asp Glu Gln Gly Glu Ala Arg Glu Ala Phe Leu Asn
        355                 360                 365

Gly Ser Asp Gly His Lys Arg Lys Glu Glu Phe Phe Ile
    370                 375                 380
```

<210> 47
<211> 388
<212> PRT
<213> Homo sapiens

<400> 47

```
Met Gly Arg Ala Arg Arg Phe Gln Trp Pro Leu Leu Leu Leu Trp Ala
1               5                  10                  15

Ala Ala Ala Gly Pro Gly Ala Gly Gln Glu Val Gln Thr Glu Asn Val
            20                  25                  30

Thr Val Ala Glu Gly Gly Val Ala Glu Ile Thr Cys Arg Leu His Gln
        35                  40                  45

Tyr Asp Gly Ser Ile Val Val Ile Gln Asn Pro Ala Arg Gln Thr Leu
    50                  55                  60

Phe Phe Asn Gly Thr Arg Ala Leu Lys Asp Glu Arg Phe Gln Leu Glu
65                  70                  75                  80

Glu Phe Ser Pro Arg Arg Val Arg Ile Arg Leu Ser Asp Ala Arg Leu
                85                  90                  95

Glu Asp Glu Gly Gly Tyr Phe Cys Gln Leu Tyr Thr Glu Asp Thr His
            100                 105                 110

His Gln Ile Ala Thr Leu Thr Val Leu Val Ala Pro Glu Asn Pro Val
        115                 120                 125

Val Glu Val Arg Glu Gln Ala Val Glu Gly Gly Glu Val Glu Leu Ser
    130                 135                 140

Cys Leu Val Pro Arg Ser Arg Pro Ala Ala Thr Leu Arg Trp Tyr Arg
145                 150                 155                 160

Asp Arg Lys Glu Leu Lys Gly Val Ser Ser Ser Gln Glu Asn Gly Lys
                165                 170                 175

Val Trp Ser Val Ala Ser Thr Val Arg Phe Arg Val Asp Arg Lys Asp
            180                 185                 190
```

Asp Gly Gly Ile Ile Ile Cys Glu Ala Gln Asn Gln Ala Leu Pro Ser
195 200 205

Gly His Ser Lys Gln Thr Gln Tyr Val Leu Asp Val Gln Tyr Ser Pro
210 215 220

Thr Ala Arg Ile His Ala Ser Gln Ala Val Val Arg Glu Gly Asp Thr
225 230 235 240

Leu Val Leu Thr Cys Ala Val Thr Gly Asn Pro Arg Pro Asn Gln Ile
245 250 255

Arg Trp Asn Arg Gly Asn Glu Ser Leu Pro Glu Arg Ala Glu Ala Val
260 265 270

Gly Glu Thr Leu Thr Leu Pro Gly Leu Val Ser Ala Asp Asn Gly Thr
275 280 285

Tyr Thr Cys Glu Ala Ser Asn Lys His Gly His Ala Arg Ala Leu Tyr
290 295 300

Val Leu Val Val Tyr Asp Pro Gly Ala Val Val Glu Ala Gln Thr Ser
305 310 315 320

Val Pro Tyr Ala Ile Val Gly Gly Ile Leu Ala Leu Leu Val Phe Leu
325 330 335

Ile Ile Cys Val Leu Val Gly Met Val Trp Cys Ser Val Arg Gln Lys
340 345 350

Gly Ser Tyr Leu Thr His Glu Ala Ser Gly Leu Asp Glu Gln Gly Glu
355 360 365

Ala Arg Glu Ala Phe Leu Asn Gly Ser Asp Gly His Lys Arg Lys Glu
370 375 380

Glu Phe Phe Ile
385

<210> 48
<211> 345
<212> PRT
<213> Homo sapiens

<400> 48

```
Met Gly Arg Ala Arg Arg Phe Gln Trp Pro Leu Leu Leu Leu Trp Ala
1               5                  10                  15

Ala Ala Ala Val Pro Gly Ala Gly Gln Glu Val Gln Thr Glu Asn Val
            20                  25                  30

Thr Val Ala Glu Gly Gly Val Ala Glu Ile Thr Cys Arg Leu His Gln
        35                  40                  45

Tyr Asp Gly Ser Ile Val Val Ile Gln Asn Pro Ala Arg Gln Thr Leu
    50                  55                  60

Phe Phe Asn Gly Thr Arg Ala Leu Lys Asp Glu Arg Phe Gln Leu Glu
65                  70                  75                  80

Glu Phe Ser Pro Arg Arg Val Arg Ile Arg Leu Ser Asp Ala Arg Leu
                85                  90                  95

Glu Asp Glu Gly Gly Tyr Phe Cys Gln Leu Tyr Thr Glu Asp Thr His
            100                 105                 110

His Gln Ile Ala Thr Leu Thr Val Leu Val Ala Pro Glu Asn Pro Val
        115                 120                 125

Val Glu Val Arg Glu Gln Ala Val Glu Gly Gly Glu Val Glu Leu Ser
    130                 135                 140

Cys Pro Val Pro Arg Ser Arg Pro Ala Ala Thr Leu Arg Trp Tyr Arg
145                 150                 155                 160

Asp Arg Lys Glu Leu Lys Gly Val Ser Ser Ser Gln Glu Asn Gly Lys
                165                 170                 175

Val Trp Ser Val Ala Ser Thr Val Arg Phe Arg Val Asp Arg Lys Asp
            180                 185                 190

Asp Gly Gly Ile Ile Ile Cys Glu Ala Gln Asn Gln Ala Leu Pro Ser
        195                 200                 205

Gly His Ser Lys Gln Thr Gln Tyr Val Leu Asp Val Gln Tyr Ser Pro
    210                 215                 220
```

```
Thr Ala Arg Ile His Ala Ser Gln Ala Val Val Arg Glu Gly Asp Thr
225                 230                 235                 240

Leu Val Leu Thr Cys Ala Val Thr Gly Asn Pro Arg Pro Asn Gln Ile
                245                 250                 255

Arg Trp Asn Arg Gly Asn Glu Ser Leu Pro Glu Arg Ala Glu Ala Val
            260                 265                 270

Gly Glu Thr Leu Thr Leu Pro Gly Leu Val Ser Ala Asp Asn Gly Thr
        275                 280                 285

Tyr Thr Cys Glu Ala Ser Asn Lys His Gly His Ala Arg Ala Leu Tyr
    290                 295                 300

Val Leu Val Val Tyr Gly Ser Tyr Leu Thr His Glu Ala Ser Gly Leu
305                 310                 315                 320

Asp Glu Gln Gly Glu Ala Arg Glu Ala Phe Leu Asn Gly Ser Asp Gly
                325                 330                 335

His Lys Arg Lys Glu Glu Phe Phe Ile
            340                 345
```

<210> 49
<211> 432
<212> PRT
<213> Homo sapiens

<400> 49

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Met Gly Ala Pro Ala Ala Ser Leu Leu Leu Leu Leu Leu Leu Phe Ala
1               5                   10                  15

Cys Cys Trp Ala Pro Gly Gly Ala Asn Leu Ser Gln Asp Gly Tyr Trp
            20                  25                  30

Gln Glu Gln Asp Leu Glu Leu Gly Thr Leu Ala Pro Leu Asp Glu Ala
        35                  40                  45

Ile Ser Ser Thr Val Trp Ser Ser Pro Asp Met Leu Ala Ser Gln Asp
    50                  55                  60

Ser Gln Pro Trp Thr Ser Asp Glu Thr Val Val Ala Gly Gly Thr Val
```

65 70 75 80

Val Leu Lys Cys Gln Val Lys Asp His Glu Asp Ser Ser Leu Gln Trp
85 90 95

Ser Asn Pro Ala Gln Gln Thr Leu Tyr Phe Gly Glu Lys Arg Ala Leu
100 105 110

Arg Asp Asn Arg Ile Gln Leu Val Thr Ser Thr Pro His Glu Leu Ser
115 120 125

Ile Ser Ile Ser Asn Val Ala Leu Ala Asp Glu Gly Glu Tyr Thr Cys
130 135 140

Ser Ile Phe Thr Met Pro Val Arg Thr Ala Lys Ser Leu Val Thr Val
145 150 155 160

Leu Gly Ile Pro Gln Lys Pro Ile Ile Thr Gly Tyr Lys Ser Ser Leu
165 170 175

Arg Glu Lys Asp Thr Ala Thr Leu Asn Cys Gln Ser Ser Gly Ser Lys
180 185 190

Pro Ala Ala Arg Leu Thr Trp Arg Lys Gly Asp Gln Glu Leu His Gly
195 200 205

Glu Pro Thr Arg Ile Gln Glu Asp Pro Asn Gly Lys Thr Phe Thr Val
210 215 220

Ser Ser Ser Val Thr Phe Gln Val Thr Arg Glu Asp Asp Gly Ala Ser
225 230 235 240

Ile Val Cys Ser Val Asn His Glu Ser Leu Lys Gly Ala Asp Arg Ser
245 250 255

Thr Ser Gln Arg Ile Glu Val Leu Tyr Thr Pro Thr Ala Met Ile Arg
260 265 270

Pro Asp Pro Pro His Pro Arg Glu Gly Gln Lys Leu Leu Leu His Cys
275 280 285

Glu Gly Arg Gly Asn Pro Val Pro Gln Gln Tyr Leu Trp Glu Lys Glu
290 295 300

```
Gly Ser Val Pro Pro Leu Lys Met Thr Gln Glu Ser Ala Leu Ile Phe
305                 310                 315                 320

Pro Phe Leu Asn Lys Ser Asp Ser Gly Thr Tyr Gly Cys Thr Ala Thr
                325                 330                 335

Ser Asn Met Gly Ser Tyr Lys Ala Tyr Tyr Thr Leu Asn Val Asn Asp
            340                 345                 350

Pro Ser Pro Val Pro Ser Ser Ser Ser Thr Tyr His Ala Ile Ile Gly
        355                 360                 365

Gly Ile Val Ala Phe Ile Val Phe Leu Leu Leu Ile Met Leu Ile Phe
    370                 375                 380

Leu Gly His Tyr Leu Ile Arg His Lys Gly Thr Tyr Leu Thr His Glu
385                 390                 395                 400

Ala Lys Gly Ser Asp Asp Ala Pro Asp Ala Asp Thr Ala Ile Ile Asn
                405                 410                 415

Ala Glu Gly Gly Gln Ser Gly Gly Asp Asp Lys Lys Glu Tyr Phe Ile
            420                 425                 430
```

<210> 50
<211> 344
<212> PRT
<213> Homo sapiens

<400> 50

```
Met Gly Val Cys Gly Tyr Leu Phe Leu Pro Trp Lys Cys Leu Val Val
1               5                   10                  15

Val Ser Leu Arg Leu Leu Phe Leu Val Pro Thr Gly Val Pro Val Arg
            20                  25                  30

Ser Gly Asp Ala Thr Phe Pro Lys Ala Met Asp Asn Val Thr Val Arg
        35                  40                  45

Gln Gly Glu Ser Ala Thr Leu Arg Cys Thr Ile Asp Asn Arg Val Thr
    50                  55                  60
```

```
Arg Val Ala Trp Leu Asn Arg Ser Thr Ile Leu Tyr Ala Gly Asn Asp
65                  70                  75                  80

Lys Trp Cys Leu Asp Pro Arg Val Val Leu Leu Ser Asn Thr Gln Thr
                85                  90                  95

Gln Tyr Ser Ile Glu Ile Gln Asn Val Asp Val Tyr Asp Glu Gly Pro
            100                 105                 110

Tyr Thr Cys Ser Val Gln Thr Asp Asn His Pro Lys Thr Ser Arg Val
        115                 120                 125

His Leu Ile Val Gln Val Ser Pro Lys Ile Val Glu Ile Ser Ser Asp
    130                 135                 140

Ile Ser Ile Asn Glu Gly Asn Asn Ile Ser Leu Thr Cys Ile Ala Thr
145                 150                 155                 160

Gly Arg Pro Glu Pro Thr Val Thr Trp Arg His Ile Ser Pro Lys Ala
                165                 170                 175

Val Gly Phe Val Ser Glu Asp Glu Tyr Leu Glu Ile Gln Gly Ile Thr
            180                 185                 190

Arg Glu Gln Ser Gly Asp Tyr Glu Cys Ser Ala Ser Asn Asp Val Ala
        195                 200                 205

Ala Pro Val Val Arg Arg Val Lys Val Thr Val Asn Tyr Pro Pro Tyr
    210                 215                 220

Ile Ser Glu Ala Lys Gly Thr Gly Val Pro Val Gly Gln Lys Gly Thr
225                 230                 235                 240

Leu Gln Cys Glu Ala Ser Ala Val Pro Ser Ala Glu Phe Gln Trp Tyr
                245                 250                 255

Lys Asp Asp Lys Arg Leu Ile Glu Gly Lys Lys Gly Val Lys Val Glu
            260                 265                 270

Asn Arg Pro Phe Leu Ser Lys Leu Ile Phe Phe Asn Val Ser Glu His
        275                 280                 285

Asp Tyr Gly Asn Tyr Thr Cys Val Ala Ser Asn Lys Leu Gly His Thr
```

290 295 300

Asn Ala Ser Ile Met Leu Phe Gly Pro Gly Ala Val Ser Glu Val Ser
305 310 315 320

Asn Gly Thr Ser Arg Arg Ala Gly Cys Val Trp Leu Leu Pro Leu Leu
325 330 335

Val Leu His Leu Leu Leu Lys Phe
340

<210> 51
<211> 798
<212> PRT
<213> Homo sapiens

<400> 51

Met Val Trp Cys Leu Gly Leu Ala Val Leu Ser Leu Val Ile Ser Gln
1 5 10 15

Gly Ala Asp Gly Arg Gly Lys Pro Glu Val Val Ser Val Val Gly Arg
20 25 30

Ala Gly Glu Ser Val Val Leu Gly Cys Asp Leu Leu Pro Pro Ala Gly
35 40 45

Arg Pro Pro Leu His Val Ile Glu Trp Leu Arg Phe Gly Phe Leu Leu
50 55 60

Pro Ile Phe Ile Gln Phe Gly Leu Tyr Ser Pro Arg Ile Asp Pro Asp
65 70 75 80

Tyr Val Gly Arg Val Arg Leu Gln Lys Gly Ala Ser Leu Gln Ile Glu
85 90 95

Gly Leu Arg Val Glu Asp Gln Gly Trp Tyr Glu Cys Arg Val Phe Phe
100 105 110

Leu Asp Gln His Ile Pro Glu Asp Asp Phe Ala Asn Gly Ser Trp Val
115 120 125

His Leu Thr Val Asn Ser Pro Pro Gln Phe Gln Glu Thr Pro Pro Ala
130 135 140

```
Val Leu Glu Val Gln Glu Leu Glu Pro Val Thr Leu Arg Cys Val Ala
145                 150                 155                 160

Arg Gly Ser Pro Leu Pro His Val Thr Trp Lys Leu Arg Gly Lys Asp
                165                 170                 175

Leu Gly Gln Gly Gln Gly Gln Val Gln Val Gln Asn Gly Thr Leu Arg
            180                 185                 190

Ile Arg Arg Val Glu Arg Gly Ser Ser Gly Val Tyr Thr Cys Gln Ala
        195                 200                 205

Ser Ser Thr Glu Gly Ser Ala Thr His Ala Thr Gln Leu Leu Val Leu
    210                 215                 220

Gly Pro Pro Val Ile Val Val Pro Pro Lys Asn Ser Thr Val Asn Ala
225                 230                 235                 240

Ser Gln Asp Val Ser Leu Ala Cys His Ala Glu Ala Tyr Pro Ala Asn
                245                 250                 255

Leu Thr Tyr Ser Trp Phe Gln Asp Asn Ile Asn Val Phe His Ile Ser
            260                 265                 270

Arg Leu Gln Pro Arg Val Arg Ile Leu Val Asp Gly Ser Leu Arg Leu
        275                 280                 285

Leu Ala Thr Gln Pro Asp Asp Ala Gly Cys Tyr Thr Cys Val Pro Ser
    290                 295                 300

Asn Gly Leu Leu His Pro Pro Ser Ala Ser Ala Tyr Leu Thr Val Leu
305                 310                 315                 320

Tyr Pro Ala Gln Val Thr Ala Met Pro Pro Glu Thr Pro Leu Pro Ile
                325                 330                 335

Gly Met Pro Gly Val Ile Arg Cys Pro Val Arg Ala Asn Pro Pro Leu
            340                 345                 350

Leu Phe Val Ser Trp Thr Lys Asp Gly Lys Ala Leu Gln Leu Asp Lys
        355                 360                 365
```

```
Phe Pro Gly Trp Ser Gln Gly Thr Glu Gly Ser Leu Ile Ile Ala Leu
    370                 375                 380

Gly Asn Glu Asp Ala Leu Gly Glu Tyr Ser Cys Thr Pro Tyr Asn Ser
385                 390                 395                 400

Leu Gly Thr Ala Gly Pro Ser Pro Val Thr Arg Val Leu Leu Lys Ala
                405                 410                 415

Pro Pro Ala Phe Ile Glu Arg Pro Lys Glu Glu Tyr Phe Gln Glu Val
            420                 425                 430

Gly Arg Glu Leu Leu Ile Pro Cys Ser Ala Gln Gly Asp Pro Pro Pro
        435                 440                 445

Val Val Ser Trp Thr Lys Val Gly Arg Gly Leu Gln Gly Gln Ala Gln
    450                 455                 460

Val Asp Ser Asn Ser Ser Leu Ile Leu Arg Pro Leu Thr Lys Glu Ala
465                 470                 475                 480

His Gly His Trp Glu Cys Ser Ala Ser Asn Ala Val Ala Arg Val Ala
                485                 490                 495

Thr Ser Thr Asn Val Tyr Val Leu Gly Thr Ser Pro His Val Val Thr
            500                 505                 510

Asn Val Ser Val Val Ala Leu Pro Lys Gly Ala Asn Val Ser Trp Glu
        515                 520                 525

Pro Gly Phe Asp Gly Gly Tyr Leu Gln Arg Phe Ser Val Trp Tyr Thr
    530                 535                 540

Pro Leu Ala Lys Arg Pro Asp Arg Met His His Asp Trp Val Ser Leu
545                 550                 555                 560

Ala Val Pro Val Gly Ala Ala His Leu Leu Val Pro Gly Leu Gln Pro
                565                 570                 575

His Thr Gln Tyr Gln Phe Ser Val Leu Ala Gln Asn Lys Leu Gly Ser
            580                 585                 590

Gly Pro Phe Ser Glu Ile Val Leu Ser Ala Pro Glu Gly Leu Pro Thr
```

595 600 605

Thr Pro Ala Ala Pro Gly Leu Pro Pro Thr Glu Ile Pro Pro Pro Leu
610 615 620

Ser Pro Pro Arg Gly Leu Val Ala Val Arg Thr Pro Arg Gly Val Leu
625 630 635 640

Leu His Trp Asp Pro Pro Glu Leu Val Pro Lys Arg Leu Asp Gly Tyr
645 650 655

Val Leu Glu Gly Arg Gln Gly Ser Gln Gly Trp Glu Val Leu Asp Pro
660 665 670

Ala Val Ala Gly Thr Glu Thr Glu Leu Leu Val Pro Gly Leu Ile Lys
675 680 685

Val Cys Ser Leu Arg Val Pro Pro Arg Gly Leu Arg Gly Gln Leu Arg
690 695 700

Gln Arg Pro Gln Gln His Gly Gln Arg Leu His Phe Arg Ser Gly Gly
705 710 715 720

Leu Pro Phe Ala His Ala Ala Ala Gly Pro Pro Ala Ser Ala Arg Ala
725 730 735

Gly Arg Arg Gly Gly Arg Ser Leu Leu Ser Gly Ser Gly Arg Pro Cys
740 745 750

Glu His Pro Gly Arg Leu Pro Pro Glu Pro Ala Gln Gly Cys Pro Pro
755 760 765

Pro Pro Gln Ala Pro Pro Pro Arg Ser Thr Ser Tyr Leu Leu Ser Asp
770 775 780

Arg Glu Val Ser Cys Thr Leu Cys Ser Gly Leu Arg Gln Ser
785 790 795

<210> 52
<211> 779
<212> PRT
<213> Homo sapiens

<400> 52

```
Gly Met Lys Pro Phe Gln Leu Asp Leu Leu Phe Val Cys Phe Phe Leu
1               5                  10                  15

Phe Ser Gln Glu Leu Gly Leu Gln Lys Arg Gly Cys Cys Leu Val Leu
            20                  25                  30

Gly Tyr Met Ala Lys Asp Lys Phe Arg Arg Met Asn Glu Gly Gln Val
        35                  40                  45

Tyr Ser Phe Ser Gln Gln Pro Gln Asp Gln Val Val Val Ser Gly Gln
    50                  55                  60

Pro Val Thr Leu Leu Cys Ala Ile Pro Glu Tyr Asp Gly Phe Val Leu
65                  70                  75                  80

Trp Ile Lys Asp Gly Leu Ala Leu Gly Val Gly Arg Asp Leu Ser Ser
                85                  90                  95

Tyr Pro Gln Tyr Leu Val Val Gly Asn His Leu Ser Gly Glu His His
            100                 105                 110

Leu Lys Ile Leu Arg Ala Glu Leu Gln Asp Asp Ala Val Tyr Glu Cys
        115                 120                 125

Gln Ala Ile Gln Ala Ala Ile Arg Ser Arg Pro Ala Arg Leu Thr Val
    130                 135                 140

Leu Val Pro Pro Asp Asp Pro Val Ile Leu Gly Gly Pro Val Ile Ser
145                 150                 155                 160

Leu Arg Ala Gly Asp Pro Leu Asn Leu Thr Cys His Ala Asp Asn Ala
                165                 170                 175

Lys Pro Ala Ala Ser Ile Ile Trp Leu Arg Lys Gly Glu Val Ile Asn
            180                 185                 190

Gly Ala Thr Tyr Ser Lys Thr Leu Leu Arg Asp Gly Lys Arg Glu Ser
        195                 200                 205

Ile Val Ser Thr Leu Phe Ile Ser Pro Gly Asp Val Glu Asn Gly Gln
    210                 215                 220
```

```
Ser Ile Val Cys Arg Ala Thr Asn Lys Ala Ile Pro Gly Gly Lys Glu
225                 230                 235                 240

Thr Ser Val Thr Ile Asp Ile Gln His Pro Pro Leu Val Asn Leu Ser
                245                 250                 255

Val Glu Pro Gln Pro Val Leu Glu Asp Asn Val Val Thr Phe His Cys
            260                 265                 270

Ser Ala Lys Ala Asn Pro Ala Val Thr Gln Tyr Arg Trp Ala Lys Arg
        275                 280                 285

Gly Gln Ile Ile Lys Glu Ala Ser Gly Glu Val Tyr Arg Thr Thr Val
    290                 295                 300

Asp Tyr Thr Tyr Phe Ser Glu Pro Val Ser Cys Glu Val Thr Asn Ala
305                 310                 315                 320

Leu Gly Ser Thr Asn Leu Ser Arg Thr Val Asp Val Tyr Phe Gly Pro
                325                 330                 335

Arg Met Thr Thr Glu Pro Gln Ser Leu Leu Val Asp Leu Gly Ser Asp
            340                 345                 350

Ala Ile Phe Ser Cys Ala Trp Thr Gly Asn Pro Ser Leu Thr Ile Val
        355                 360                 365

Trp Met Lys Arg Gly Ser Gly Val Val Leu Ser Asn Glu Lys Thr Leu
    370                 375                 380

Thr Leu Lys Ser Val Arg Gln Glu Asp Ala Gly Lys Tyr Val Cys Arg
385                 390                 395                 400

Ala Val Val Pro Arg Val Gly Ala Gly Glu Arg Glu Val Thr Leu Thr
                405                 410                 415

Val Asn Gly Pro Pro Ile Ile Ser Ser Thr Gln Thr Gln His Ala Leu
            420                 425                 430

His Gly Glu Lys Gly Gln Ile Lys Cys Phe Ile Arg Ser Thr Pro Pro
        435                 440                 445

Pro Asp Arg Ile Ala Trp Ser Trp Lys Glu Asn Val Leu Glu Ser Gly
```

```
    450                 455                 460

Thr Ser Gly Arg Tyr Thr Val Glu Thr Ile Ser Thr Glu Glu Gly Val
465                 470                 475                 480

Ile Ser Thr Leu Thr Ile Ser Asn Ile Val Arg Ala Asp Phe Gln Thr
                485                 490                 495

Ile Tyr Asn Cys Thr Ala Trp Asn Ser Phe Gly Ser Asp Thr Glu Ile
            500                 505                 510

Ile Arg Leu Lys Glu Gln Gly Ser Glu Met Lys Ser Gly Ala Gly Leu
        515                 520                 525

Glu Ala Glu Ser Val Pro Met Ala Val Ile Ile Gly Val Ala Val Gly
    530                 535                 540

Ala Gly Val Ala Phe Leu Val Leu Met Ala Thr Ile Val Ala Phe Cys
545                 550                 555                 560

Cys Ala Arg Ser Gln Arg Asn Leu Lys Gly Val Val Ser Ala Lys Asn
                565                 570                 575

Asp Ile Arg Val Glu Ile Val His Lys Glu Pro Ala Ser Gly Arg Glu
            580                 585                 590

Gly Glu Glu His Ser Thr Ile Lys Gln Leu Met Met Asp Arg Gly Glu
        595                 600                 605

Phe Gln Gln Asp Ser Val Leu Lys Gln Leu Glu Val Leu Lys Glu Glu
    610                 615                 620

Glu Lys Glu Phe Gln Asn Leu Lys Asp Pro Thr Asn Gly Tyr Tyr Ser
625                 630                 635                 640

Val Asn Thr Phe Lys Glu His His Ser Thr Pro Thr Ile Ser Leu Ser
                645                 650                 655

Ser Cys Gln Pro Asp Leu Arg Pro Ala Gly Lys Gln Arg Val Pro Thr
            660                 665                 670

Gly Met Ser Phe Thr Asn Ile Tyr Ser Thr Leu Ser Gly Gln Gly Arg
        675                 680                 685
```

```
Leu Tyr Asp Tyr Gly Gln Arg Phe Val Leu Gly Met Gly Ser Ser Ser
    690                 695                 700

Ile Glu Leu Cys Glu Arg Glu Phe Gln Arg Gly Ser Leu Ser Asp Ser
705                 710                 715                 720

Ser Ser Phe Leu Asp Thr Gln Cys Asp Ser Ser Val Ser Ser Ser Gly
                725                 730                 735

Lys Gln Asp Gly Tyr Val Gln Phe Asp Lys Ala Ser Lys Ala Ser Ala
            740                 745                 750

Ser Ser Ser His His Ser Gln Ser Ser Ser Gln Asn Ser Asp Pro Ser
        755                 760                 765

Arg Pro Leu Gln Arg Arg Met Gln Thr His Val
    770                 775
```

<210> 53
<211> 442
<212> PRT
<213> Homo sapiens

<400> 53

```
Met Thr Thr Glu Pro Gln Ser Leu Leu Val Asp Leu Gly Ser Asp Ala
1               5                   10                  15

Ile Phe Ser Cys Ala Trp Thr Gly Asn Pro Ser Leu Thr Ile Val Trp
            20                  25                  30

Met Lys Arg Gly Ser Gly Val Val Leu Ser Asn Glu Lys Thr Leu Thr
        35                  40                  45

Leu Lys Ser Val Arg Gln Glu Asp Ala Gly Lys Tyr Val Cys Arg Ala
    50                  55                  60

Val Val Pro Arg Val Gly Ala Gly Glu Arg Glu Val Thr Leu Thr Val
65                  70                  75                  80

Asn Gly Pro Pro Ile Ile Ser Ser Thr Gln Thr Gln His Ala Leu His
                85                  90                  95
```

```
Gly Glu Lys Gly Gln Ile Lys Cys Phe Ile Arg Ser Thr Pro Pro Pro
            100                 105                 110

Asp Arg Ile Ala Trp Ser Trp Lys Glu Asn Val Leu Glu Ser Gly Thr
        115                 120                 125

Ser Gly Arg Tyr Thr Val Glu Thr Ile Ser Thr Glu Glu Gly Val Ile
    130                 135                 140

Ser Thr Leu Thr Ile Ser Asn Ile Val Arg Ala Asp Phe Gln Thr Ile
145                 150                 155                 160

Tyr Asn Cys Thr Ala Trp Asn Ser Phe Gly Ser Asp Thr Glu Ile Ile
                165                 170                 175

Arg Leu Lys Glu Gln Gly Ser Glu Met Lys Ser Gly Ala Gly Leu Glu
            180                 185                 190

Ala Glu Ser Val Pro Met Ala Val Ile Ile Gly Val Ala Val Gly Ala
        195                 200                 205

Gly Val Ala Phe Leu Val Leu Met Ala Thr Ile Val Ala Phe Cys Cys
    210                 215                 220

Ala Arg Ser Gln Arg Asn Leu Lys Gly Val Val Ser Ala Lys Asn Asp
225                 230                 235                 240

Ile Arg Val Glu Ile Val His Lys Glu Pro Ala Ser Gly Arg Glu Gly
                245                 250                 255

Glu Glu His Ser Thr Ile Lys Gln Leu Met Met Asp Arg Gly Glu Phe
            260                 265                 270

Gln Gln Asp Ser Val Leu Lys Gln Leu Glu Val Leu Lys Glu Glu Glu
        275                 280                 285

Lys Glu Phe Gln Asn Leu Lys Asp Pro Thr Asn Gly Tyr Tyr Ser Val
    290                 295                 300

Asn Thr Phe Lys Glu His His Ser Thr Pro Thr Ile Ser Leu Ser Ser
305                 310                 315                 320

Cys Gln Pro Asp Leu Arg Pro Ala Gly Lys Gln Arg Val Pro Thr Gly
```

325 330 335

Met Ser Phe Thr Asn Ile Tyr Ser Thr Leu Ser Gly Gln Gly Arg Leu
340 345 350

Tyr Asp Tyr Gly Gln Arg Phe Val Leu Gly Met Gly Ser Ser Ser Ile
355 360 365

Glu Leu Cys Glu Arg Glu Phe Gln Arg Gly Ser Leu Ser Asp Ser Ser
370 375 380

Ser Phe Leu Asp Thr Gln Cys Asp Ser Ser Val Ser Ser Ser Gly Lys
385 390 395 400

Gln Asp Gly Tyr Val Gln Phe Asp Lys Ala Ser Lys Ala Ser Ala Ser
405 410 415

Ser Ser His His Ser Gln Ser Ser Ser Gln Asn Ser Asp Pro Ser Arg
420 425 430

Pro Leu Gln Arg Arg Met Gln Thr His Val
435 440

<210> 54
<211> 510
<212> PRT
<213> Homo sapiens

<400> 54

Met Pro Leu Ser Leu Gly Ala Glu Met Trp Gly Pro Glu Ala Trp Leu
1 5 10 15

Leu Leu Leu Leu Leu Leu Ala Ser Phe Thr Gly Arg Cys Pro Ala Gly
20 25 30

Glu Leu Glu Thr Ser Asp Val Val Thr Val Val Leu Gly Gln Asp Ala
35 40 45

Lys Leu Pro Cys Phe Tyr Arg Gly Asp Ser Gly Glu Gln Val Gly Gln
50 55 60

Val Ala Trp Ala Arg Val Asp Ala Gly Glu Gly Ala Gln Glu Leu Ala
65 70 75 80

```
Leu Leu His Ser Lys Tyr Gly Leu His Val Ser Pro Ala Tyr Glu Gly
                85                  90                  95

Arg Val Glu Gln Pro Pro Pro Pro Arg Asn Pro Leu Asp Gly Ser Val
            100                 105                 110

Leu Leu Arg Asn Ala Val Gln Ala Asp Glu Gly Glu Tyr Glu Cys Arg
        115                 120                 125

Val Ser Thr Phe Pro Ala Gly Ser Phe Gln Ala Arg Leu Arg Leu Arg
    130                 135                 140

Val Met Val Pro Pro Leu Pro Ser Leu Asn Pro Gly Pro Ala Leu Glu
145                 150                 155                 160

Glu Gly Gln Gly Leu Thr Leu Ala Ala Ser Cys Thr Ala Glu Gly Ser
                165                 170                 175

Pro Ala Pro Ser Val Thr Trp Asp Thr Glu Val Lys Gly Thr Thr Ser
            180                 185                 190

Ser Arg Ser Phe Lys His Ser Arg Ser Ala Ala Val Thr Ser Glu Phe
        195                 200                 205

His Leu Val Pro Ser Arg Ser Met Asn Gly Gln Pro Leu Thr Cys Val
    210                 215                 220

Val Ser His Pro Gly Leu Leu Gln Asp Gln Arg Ile Thr His Ile Leu
225                 230                 235                 240

His Val Ser Phe Leu Ala Glu Ala Ser Val Arg Gly Leu Glu Asp Gln
                245                 250                 255

Asn Leu Trp His Ile Gly Arg Glu Gly Ala Met Leu Lys Cys Leu Ser
            260                 265                 270

Glu Gly Gln Pro Pro Pro Ser Tyr Asn Trp Thr Arg Leu Asp Gly Pro
        275                 280                 285

Leu Pro Ser Gly Val Arg Val Asp Gly Asp Thr Leu Gly Phe Pro Pro
    290                 295                 300
```

```
Leu Thr Thr Glu His Ser Gly Ile Tyr Val Cys His Val Ser Asn Glu
305                 310                 315                 320

Phe Ser Ser Arg Asp Ser Gln Val Thr Val Asp Val Leu Asp Pro Gln
                325                 330                 335

Glu Asp Ser Gly Lys Gln Val Asp Leu Val Ser Ala Ser Val Val Val
            340                 345                 350

Val Gly Val Ile Ala Ala Leu Leu Phe Cys Leu Leu Val Val Val Val
        355                 360                 365

Val Leu Met Ser Arg Tyr His Arg Arg Lys Ala Gln Gln Met Thr Gln
    370                 375                 380

Lys Tyr Glu Glu Glu Leu Thr Leu Thr Arg Glu Asn Ser Ile Arg Arg
385                 390                 395                 400

Leu His Ser His His Thr Asp Pro Arg Ser Gln Pro Glu Glu Ser Val
                405                 410                 415

Gly Leu Arg Ala Glu Gly His Pro Asp Ser Leu Lys Asp Asn Ser Ser
            420                 425                 430

Cys Ser Val Met Ser Glu Glu Pro Glu Gly Arg Ser Tyr Ser Thr Leu
        435                 440                 445

Thr Thr Val Arg Glu Ile Glu Thr Gln Thr Glu Leu Leu Ser Pro Gly
    450                 455                 460

Ser Gly Arg Ala Glu Glu Glu Glu Asp Gln Asp Glu Gly Ile Lys Gln
465                 470                 475                 480

Ala Met Asn His Phe Val Gln Glu Asn Gly Thr Leu Arg Ala Lys Pro
                485                 490                 495

Thr Gly Asn Gly Ile Tyr Ile Asn Gly Arg Gly His Leu Val
            500                 505                 510
```

<210> 55
<211> 510
<212> PRT
<213> Homo sapiens

<400> 55

```
Met Pro Leu Ser Leu Gly Ala Glu Met Trp Gly Pro Glu Ala Trp Leu
1               5                  10                  15

Leu Leu Leu Leu Leu Leu Ala Ser Phe Thr Gly Arg Cys Pro Ala Gly
            20                  25                  30

Glu Leu Gly Thr Ser Asp Val Val Thr Val Val Leu Gly Gln Asp Ala
        35                  40                  45

Lys Leu Pro Cys Phe Tyr Arg Gly Asp Ser Gly Glu Gln Val Gly Gln
    50                  55                  60

Val Ala Trp Ala Arg Val Asp Ala Gly Glu Gly Ala Gln Glu Leu Ala
65                  70                  75                  80

Leu Leu His Ser Lys Tyr Gly Leu His Val Ser Pro Ala Tyr Glu Gly
                85                  90                  95

Arg Val Glu Gln Pro Pro Pro Pro Arg Asn Pro Leu Asp Gly Ser Val
            100                 105                 110

Leu Leu Arg Asn Ala Val Gln Ala Asp Glu Gly Glu Tyr Glu Cys Arg
        115                 120                 125

Val Ser Thr Phe Pro Ala Gly Ser Phe Gln Ala Arg Leu Arg Leu Arg
    130                 135                 140

Val Leu Val Pro Pro Leu Pro Ser Leu Asn Pro Gly Pro Ala Leu Glu
145                 150                 155                 160

Glu Gly Gln Gly Leu Thr Leu Ala Ala Ser Cys Thr Ala Glu Gly Ser
                165                 170                 175

Pro Ala Pro Ser Val Thr Trp Asp Thr Glu Val Lys Gly Thr Thr Ser
            180                 185                 190

Ser Arg Ser Phe Lys His Ser Arg Ser Ala Ala Val Thr Ser Glu Phe
        195                 200                 205

His Leu Val Pro Ser Arg Ser Met Asn Gly Gln Pro Leu Thr Cys Val
    210                 215                 220
```

```
Val Ser His Pro Gly Leu Leu Gln Asp Gln Arg Ile Thr His Ile Leu
225                 230                 235                 240

His Val Ser Phe Leu Ala Glu Ala Ser Val Arg Gly Leu Glu Asp Gln
                245                 250                 255

Asn Leu Trp His Ile Gly Arg Glu Gly Ala Met Leu Lys Cys Leu Ser
            260                 265                 270

Glu Gly Gln Pro Pro Pro Ser Tyr Asn Trp Thr Arg Leu Asp Gly Pro
        275                 280                 285

Leu Pro Ser Gly Val Arg Val Asp Gly Asp Thr Leu Gly Phe Pro Pro
    290                 295                 300

Leu Thr Thr Glu His Ser Gly Ile Tyr Val Cys His Val Ser Asn Glu
305                 310                 315                 320

Phe Ser Ser Arg Asp Ser Gln Val Thr Val Asp Val Leu Asp Pro Gln
                325                 330                 335

Glu Asp Ser Gly Lys Gln Val Asp Leu Val Ser Ala Ser Val Val Val
            340                 345                 350

Val Gly Val Ile Ala Ala Leu Leu Phe Cys Leu Leu Val Val Val Val
        355                 360                 365

Val Leu Met Ser Arg Tyr His Arg Arg Lys Ala Gln Gln Met Thr Gln
    370                 375                 380

Lys Tyr Glu Glu Glu Leu Thr Leu Thr Arg Glu Asn Ser Ile Arg Arg
385                 390                 395                 400

Leu His Ser His His Thr Asp Pro Arg Ser Gln Pro Glu Glu Ser Val
                405                 410                 415

Gly Leu Arg Ala Glu Gly His Pro Asp Ser Leu Lys Asp Asn Ser Ser
            420                 425                 430

Cys Ser Val Met Ser Glu Glu Pro Glu Gly Arg Ser Tyr Ser Thr Leu
        435                 440                 445
```

```
Thr Thr Val Arg Glu Ile Glu Thr Gln Thr Glu Leu Leu Ser Pro Gly
    450                 455                 460

Ser Gly Arg Ala Glu Glu Glu Glu Asp Gln Asp Glu Gly Ile Lys Gln
465                 470                 475                 480

Ala Met Asn His Phe Val Gln Glu Asn Gly Thr Leu Arg Ala Lys Pro
                485                 490                 495

Thr Gly Asn Gly Ile Tyr Ile Asn Gly Arg Gly His Leu Val
            500                 505                 510
```

<210> 56
<211> 348
<212> PRT
<213> Rattus norvegicus

<400> 56

```
Met Val Leu Leu Ala Gln Gly Ala Cys Cys Ser Asn Gln Trp Leu Ala
1               5                   10                  15

Ala Val Leu Leu Ser Leu Cys Ser Cys Leu Pro Ala Gly Gln Ser Val
            20                  25                  30

Asp Phe Pro Trp Ala Ala Val Asp Asn Met Leu Val Arg Lys Gly Asp
        35                  40                  45

Thr Ala Val Leu Arg Cys Tyr Leu Glu Asp Gly Ala Ser Lys Gly Ala
    50                  55                  60

Trp Leu Asn Arg Ser Ser Ile Ile Phe Ala Gly Gly Asp Lys Trp Ser
65                  70                  75                  80

Val Asp Pro Arg Val Ser Ile Ser Thr Leu Asn Lys Arg Asp Tyr Ser
                85                  90                  95

Leu Gln Ile Gln Asn Val Asp Val Thr Asp Asp Gly Pro Tyr Thr Cys
            100                 105                 110

Ser Val Gln Thr Gln His Thr Pro Arg Thr Met Gln Val His Leu Thr
        115                 120                 125

Val Gln Val Pro Pro Lys Ile Tyr Asp Ile Ser Asn Asp Met Thr Ile
    130                 135                 140
```

```
Asn Glu Gly Thr Asn Val Thr Leu Thr Cys Leu Ala Thr Gly Lys Pro
145                 150                 155                 160

Glu Pro Ala Ile Ser Trp Arg His Ile Ser Pro Ser Ala Lys Pro Phe
                165                 170                 175

Glu Asn Gly Gln Tyr Leu Asp Ile Tyr Gly Ile Thr Arg Asp Gln Ala
            180                 185                 190

Gly Glu Tyr Glu Cys Ser Ala Glu Asn Asp Val Ser Phe Pro Asp Val
        195                 200                 205

Lys Lys Val Arg Val Val Val Asn Phe Ala Pro Thr Ile Gln Glu Ile
    210                 215                 220

Lys Ser Gly Thr Val Thr Pro Gly Arg Ser Gly Leu Ile Arg Cys Glu
225                 230                 235                 240

Gly Ala Gly Val Pro Pro Pro Ala Phe Glu Trp Tyr Lys Gly Glu Lys
                245                 250                 255

Arg Leu Phe Asn Gly Gln Gln Gly Ile Ile Ile Gln Asn Phe Ser Thr
            260                 265                 270

Arg Ser Ile Leu Thr Val Thr Asn Val Thr Gln Glu His Phe Gly Asn
        275                 280                 285

Tyr Thr Cys Val Ala Ala Asn Lys Leu Gly Thr Thr Asn Ala Ser Leu
    290                 295                 300

Pro Leu Asn Pro Pro Ser Thr Ala Gln Tyr Gly Ile Thr Gly Ser Ala
305                 310                 315                 320

Cys Asp Leu Phe Ser Cys Trp Ser Leu Ala Leu Thr Leu Ser Ser Val
                325                 330                 335

Ile Ser Ile Phe Tyr Leu Lys Asn Ala Ile Leu Gln
            340                 345
```

<210> 57
<211> 348
<212> PRT

<213> Rattus norvegicus

<400> 57

```
Met Val Leu Leu Ala Gln Gly Ala Cys Cys Ser Asn Gln Trp Leu Ala
1               5                  10                  15

Ala Val Leu Leu Ser Leu Cys Ser Cys Leu Pro Ala Gly Gln Ser Val
            20                  25                  30

Asp Phe Pro Trp Ala Ala Val Asp Asn Met Leu Val Arg Lys Gly Asp
        35                  40                  45

Thr Ala Val Leu Arg Cys Tyr Leu Glu Asp Gly Ala Ser Lys Gly Ala
    50                  55                  60

Trp Leu Asn Arg Ser Ser Ile Ile Phe Ala Gly Gly Asp Lys Trp Ser
65                  70                  75                  80

Val Asp Pro Arg Val Ser Ile Ser Thr Leu Asn Lys Arg Asp Tyr Ser
                85                  90                  95

Leu Gln Ile Gln Asn Val Asp Val Thr Asp Asp Gly Pro Tyr Thr Cys
            100                 105                 110

Ser Val Gln Thr Gln His Thr Pro Arg Thr Met Gln Val His Leu Thr
        115                 120                 125

Val Gln Val Pro Pro Lys Ile Tyr Asp Ile Ser Asn Asp Met Thr Ile
    130                 135                 140

Asn Glu Gly Thr Asn Val Thr Leu Thr Cys Leu Ala Thr Gly Lys Pro
145                 150                 155                 160

Glu Pro Ala Ile Ser Trp Arg His Ile Ser Pro Ser Ala Lys Pro Phe
                165                 170                 175

Glu Asn Gly Gln Tyr Leu Asp Ile Tyr Gly Ile Thr Arg Asp Gln Ala
            180                 185                 190

Gly Glu Tyr Glu Cys Ser Ala Glu Asn Asp Val Ser Phe Pro Asp Val
        195                 200                 205

Lys Lys Val Arg Val Val Val Asn Phe Ala Pro Thr Ile Gln Glu Ile
```

```
    210                 215                 220

Lys Ser Gly Thr Val Thr Pro Gly Arg Ser Gly Leu Ile Arg Cys Glu
225                 230                 235                 240

Gly Ala Gly Val Pro Pro Pro Ala Phe Glu Trp Tyr Lys Gly Glu Lys
                245                 250                 255

Arg Leu Phe Asn Gly Gln Gln Gly Ile Ile Ile Gln Asn Phe Ser Thr
            260                 265                 270

Arg Ser Ile Leu Thr Val Thr Asn Val Thr Gln Glu His Phe Gly Asn
        275                 280                 285

Tyr Thr Cys Val Ala Ala Asn Lys Leu Gly Thr Thr Asn Ala Ser Leu
    290                 295                 300

Pro Leu Asn Pro Pro Ser Thr Ala Gln Tyr Gly Ile Thr Gly Ser Ala
305                 310                 315                 320

Cys Asp Leu Phe Ser Cys Trp Ser Leu Ala Leu Thr Leu Ser Ser Val
                325                 330                 335

Ile Ser Ile Phe Tyr Leu Lys Asn Ala Ile Leu Gln
            340                 345
```

<210> 58
<211> 338
<212> PRT
<213> Homo sapiens

<400> 58

```
Met Val Gly Arg Val Gln Pro Asp Arg Lys Gln Leu Pro Leu Val Leu
1               5                   10                  15

Leu Arg Leu Leu Cys Leu Leu Pro Thr Gly Leu Pro Val Arg Ser Val
            20                  25                  30

Asp Phe Asn Arg Gly Thr Asp Asn Ile Thr Val Arg Gln Gly Asp Thr
        35                  40                  45

Ala Ile Leu Arg Cys Val Leu Glu Asp Lys Asn Ser Lys Val Ala Trp
    50                  55                  60
```

```
Leu Asn Arg Ser Gly Ile Ile Phe Ala Gly His Asp Lys Trp Ser Leu
65                  70                  75                  80

Asp Pro Arg Val Glu Leu Glu Lys Arg His Ser Leu Glu Tyr Ser Leu
                85                  90                  95

Arg Ile Gln Lys Val Asp Val Tyr Asp Glu Gly Ser Tyr Thr Cys Ser
            100                 105                 110

Val Gln Thr Gln His Glu Pro Lys Thr Ser Gln Val Tyr Leu Ile Val
        115                 120                 125

Gln Val Pro Pro Lys Ile Ser Asn Ile Ser Ser Asp Val Thr Val Asn
    130                 135                 140

Glu Gly Ser Asn Val Thr Leu Val Cys Met Ala Asn Gly Arg Pro Glu
145                 150                 155                 160

Pro Val Ile Thr Trp Arg His Leu Thr Pro Thr Gly Arg Glu Phe Glu
                165                 170                 175

Gly Glu Glu Glu Tyr Leu Glu Ile Leu Gly Ile Thr Arg Glu Gln Ser
            180                 185                 190

Gly Lys Tyr Glu Cys Lys Ala Ala Asn Glu Val Ser Ser Ala Asp Val
        195                 200                 205

Lys Gln Val Lys Val Thr Val Asn Tyr Pro Pro Thr Ile Thr Glu Ser
    210                 215                 220

Lys Ser Asn Glu Ala Thr Thr Gly Arg Gln Ala Ser Leu Lys Cys Glu
225                 230                 235                 240

Ala Ser Ala Val Pro Ala Pro Asp Phe Glu Trp Tyr Arg Asp Asp Thr
                245                 250                 255

Arg Ile Asn Ser Ala Asn Gly Leu Glu Ile Lys Ser Thr Glu Gly Gln
            260                 265                 270

Ser Ser Leu Thr Val Thr Asn Val Thr Glu Glu His Tyr Gly Asn Tyr
        275                 280                 285
```

```
Thr Cys Val Ala Ala Asn Lys Leu Gly Val Thr Asn Ala Ser Leu Val
    290                 295                 300

Leu Phe Arg Pro Gly Ser Val Arg Gly Ile Asn Gly Ser Ile Ser Leu
305                 310                 315                 320

Ala Val Pro Leu Trp Leu Leu Ala Ala Ser Leu Leu Cys Leu Leu Ser
                325                 330                 335

Lys Cys
```

<210> 59
<211> 345
<212> PRT
<213> Homo sapiens

<400> 59

```
Met Gly Val Cys Gly Tyr Leu Phe Leu Pro Trp Lys Cys Leu Val Val
1               5                   10                  15

Val Ser Leu Arg Leu Leu Phe Leu Val Pro Thr Gly Val Pro Val Arg
            20                  25                  30

Ser Gly Asp Ala Thr Phe Pro Lys Ala Met Asp Asn Val Thr Val Arg
        35                  40                  45

Gln Gly Glu Ser Ala Thr Leu Arg Cys Thr Ile Asp Asp Arg Val Thr
    50                  55                  60

Arg Val Ala Trp Leu Asn Arg Ser Thr Ile Leu Tyr Ala Gly Asn Asp
65                  70                  75                  80

Lys Trp Ser Ile Asp Pro Arg Val Ile Ile Leu Val Asn Thr Pro Thr
                85                  90                  95

Gln Tyr Ser Ile Met Ile Gln Asn Val Asp Val Tyr Asp Glu Gly Pro
            100                 105                 110

Tyr Thr Cys Ser Val Gln Thr Asp Asn His Pro Lys Thr Ser Arg Val
        115                 120                 125

His Leu Ile Val Gln Val Pro Pro Gln Ile Met Asn Ile Ser Ser Asp
    130                 135                 140
```

```
Ile Thr Val Asn Glu Gly Ser Ser Val Thr Leu Leu Cys Leu Ala Ile
145                 150                 155                 160

Gly Arg Pro Glu Pro Thr Val Thr Trp Arg His Leu Ser Val Lys Glu
                165                 170                 175

Gly Gln Gly Phe Val Ser Glu Asp Glu Tyr Leu Glu Ile Ser Asp Ile
            180                 185                 190

Lys Arg Asp Gln Ser Gly Glu Tyr Glu Cys Ser Ala Leu Asn Asp Val
        195                 200                 205

Ala Ala Pro Asp Val Arg Lys Val Lys Ile Thr Val Asn Tyr Pro Pro
    210                 215                 220

Tyr Ile Ser Lys Ala Lys Asn Thr Gly Val Ser Val Gly Gln Lys Gly
225                 230                 235                 240

Ile Leu Ser Cys Glu Ala Ser Ala Val Pro Met Ala Glu Phe Gln Trp
                245                 250                 255

Phe Lys Glu Glu Thr Arg Leu Ala Thr Gly Leu Asp Gly Met Arg Ile
            260                 265                 270

Glu Asn Lys Gly Arg Met Ser Thr Leu Thr Phe Phe Asn Val Ser Glu
        275                 280                 285

Lys Asp Tyr Gly Asn Tyr Thr Cys Val Ala Thr Asn Lys Leu Gly Asn
    290                 295                 300

Thr Asn Ala Ser Ile Thr Leu Tyr Gly Pro Gly Ala Val Ile Asp Gly
305                 310                 315                 320

Val Asn Ser Ala Ser Arg Ala Leu Ala Cys Leu Trp Leu Ser Gly Thr
                325                 330                 335

Leu Leu Ala His Phe Phe Ile Lys Phe
            340                 345
```

<210> 60
<211> 514
<212> PRT

<213> Homo sapiens

<400> 60

Met Gly Leu Ala Gly Ala Ala Gly Arg Trp Trp Gly Leu Ala Leu Gly
1 5 10 15

Leu Thr Ala Phe Phe Leu Pro Gly Val His Ser Gln Val Val Gln Val
20 25 30

Asn Asp Ser Met Tyr Gly Phe Ile Gly Thr Asp Val Val Leu His Cys
35 40 45

Ser Phe Ala Asn Pro Leu Pro Ser Val Lys Ile Thr Gln Val Thr Trp
50 55 60

Gln Lys Ser Thr Asn Gly Ser Lys Gln Asn Val Ala Ile Tyr Asn Pro
65 70 75 80

Ser Met Gly Val Ser Val Leu Ala Pro Tyr Arg Glu Arg Val Glu Phe
85 90 95

Leu Arg Pro Ser Phe Thr Asp Gly Thr Ile Arg Leu Ser Arg Leu Glu
100 105 110

Leu Glu Asp Glu Gly Val Tyr Ile Cys Glu Phe Ala Thr Phe Pro Thr
115 120 125

Gly Asn Arg Glu Ser Gln Leu Asn Leu Thr Val Met Ala Lys Pro Thr
130 135 140

Asn Trp Ile Glu Gly Thr Gln Ala Val Leu Arg Ala Lys Lys Gly Gln
145 150 155 160

Asp Asp Lys Val Leu Val Ala Thr Cys Thr Ser Ala Asn Gly Lys Pro
165 170 175

Pro Ser Val Val Ser Trp Glu Thr Arg Leu Lys Gly Glu Ala Glu Tyr
180 185 190

Gln Glu Ile Arg Asn Pro Asn Gly Thr Val Thr Val Ile Ser Arg Tyr
195 200 205

Arg Leu Val Pro Ser Arg Glu Ala His Gln Gln Ser Leu Ala Cys Ile

```
    210                 215                 220

Val Asn Tyr His Met Asp Arg Phe Lys Glu Ser Leu Thr Leu Asn Val
225                 230                 235                 240

Gln Tyr Glu Pro Glu Val Thr Ile Glu Gly Phe Asp Gly Asn Trp Tyr
                245                 250                 255

Leu Gln Arg Met Asp Val Lys Leu Thr Cys Lys Ala Asp Ala Asn Pro
            260                 265                 270

Pro Ala Thr Glu Tyr His Trp Thr Thr Leu Asn Gly Ser Leu Pro Lys
        275                 280                 285

Gly Val Glu Ala Gln Asn Arg Thr Leu Phe Phe Lys Gly Pro Ile Asn
    290                 295                 300

Tyr Ser Leu Ala Gly Thr Tyr Ile Cys Glu Ala Thr Asn Pro Ile Gly
305                 310                 315                 320

Thr Arg Ser Gly Gln Val Glu Val Asn Ile Thr Glu Phe Pro Tyr Thr
                325                 330                 335

Pro Ser Pro Pro Glu His Gly Arg Arg Ala Gly Pro Val Pro Thr Ala
            340                 345                 350

Ile Ile Gly Gly Val Ala Gly Ser Ile Leu Leu Val Leu Ile Val Val
        355                 360                 365

Gly Gly Ile Val Val Ala Leu Arg Arg Arg Arg His Thr Phe Lys Gly
    370                 375                 380

Asp Tyr Ser Thr Lys Lys His Val Tyr Gly Asn Gly Tyr Ser Lys Ala
385                 390                 395                 400

Gly Ile Pro Gln His His Pro Pro Met Ala Gln Asn Leu Gln Tyr Pro
                405                 410                 415

Asp Asp Ser Asp Asp Glu Lys Lys Ala Gly Pro Leu Gly Gly Ser Ser
            420                 425                 430

Tyr Glu Glu Glu Glu Glu Glu Glu Glu Gly Gly Gly Gly Gly Glu Arg
        435                 440                 445
```

```
Lys Val Gly Gly Pro His Pro Lys Tyr Asp Glu Asp Ala Lys Arg Pro
    450                 455                 460

Tyr Phe Thr Val Asp Glu Ala Glu Ala Arg Gln Asp Gly Tyr Gly Asp
465                 470                 475                 480

Arg Thr Leu Gly Tyr Gln Tyr Asp Pro Glu Gln Leu Asp Leu Ala Glu
                485                 490                 495

Asn Met Val Ser Gln Asn Asp Gly Ser Phe Ile Ser Lys Lys Glu Trp
            500                 505                 510

Tyr Val
```

<210> 61
<211> 538
<212> PRT
<213> Homo sapiens

<400> 61

```
Met Ala Arg Ala Ala Ala Leu Leu Pro Ser Arg Ser Pro Pro Thr Pro
1               5                   10                  15

Leu Leu Trp Pro Leu Leu Leu Leu Leu Leu Leu Glu Thr Gly Ala Gln
            20                  25                  30

Asp Val Arg Val Gln Val Leu Pro Glu Val Arg Gly Gln Leu Gly Gly
        35                  40                  45

Thr Val Glu Leu Pro Cys His Leu Leu Pro Pro Val Pro Gly Leu Tyr
    50                  55                  60

Ile Ser Leu Val Thr Trp Gln Arg Pro Asp Ala Pro Ala Asn His Gln
65                  70                  75                  80

Asn Val Ala Ala Phe His Pro Lys Met Gly Pro Ser Phe Pro Ser Pro
                85                  90                  95

Lys Pro Gly Ser Glu Arg Leu Ser Phe Val Ser Ala Lys Gln Ser Thr
            100                 105                 110
```

```
Gly Gln Asp Thr Glu Ala Glu Leu Gln Asp Ala Thr Leu Ala Leu His
        115                 120                 125

Gly Leu Thr Val Glu Asp Glu Gly Asn Tyr Thr Cys Glu Phe Ala Thr
    130                 135                 140

Phe Pro Lys Gly Ser Val Arg Gly Met Thr Trp Leu Arg Val Ile Ala
145                 150                 155                 160

Lys Pro Lys Asn Gln Ala Glu Ala Gln Lys Val Thr Phe Ser Gln Asp
                165                 170                 175

Pro Thr Thr Val Ala Leu Cys Ile Ser Lys Glu Gly Arg Pro Pro Ala
            180                 185                 190

Arg Ile Ser Trp Leu Ser Ser Leu Asp Trp Glu Ala Lys Glu Thr Gln
        195                 200                 205

Val Ser Gly Thr Leu Ala Gly Thr Val Thr Val Thr Ser Arg Phe Thr
    210                 215                 220

Leu Val Pro Ser Gly Arg Ala Asp Gly Val Thr Val Thr Cys Lys Val
225                 230                 235                 240

Glu His Glu Ser Phe Glu Glu Pro Ala Leu Ile Pro Val Thr Leu Ser
                245                 250                 255

Val Arg Tyr Pro Pro Glu Val Ser Ile Ser Gly Tyr Asp Asp Asn Trp
            260                 265                 270

Tyr Leu Gly Arg Thr Asp Ala Thr Leu Ser Cys Asp Val Arg Ser Asn
        275                 280                 285

Pro Glu Pro Thr Gly Tyr Asp Trp Ser Thr Thr Ser Gly Thr Phe Pro
    290                 295                 300

Thr Ser Ala Val Ala Gln Gly Ser Gln Leu Val Ile His Ala Val Asp
305                 310                 315                 320

Ser Leu Phe Asn Thr Thr Phe Val Cys Thr Val Thr Asn Ala Val Gly
                325                 330                 335

Met Gly Arg Ala Glu Gln Val Ile Phe Val Arg Glu Thr Pro Asn Thr
```

340 345 350

Ala Gly Ala Gly Ala Thr Gly Gly Ile Ile Gly Gly Ile Ile Ala Ala
355 360 365

Ile Ile Ala Thr Ala Val Ala Ala Thr Gly Ile Leu Ile Cys Arg Gln
370 375 380

Gln Arg Lys Glu Gln Thr Leu Gln Gly Ala Glu Glu Asp Glu Asp Leu
385 390 395 400

Glu Gly Pro Pro Ser Tyr Lys Pro Pro Thr Pro Lys Ala Lys Leu Glu
405 410 415

Ala Gln Glu Met Pro Ser Gln Leu Phe Thr Leu Gly Ala Ser Glu His
420 425 430

Ser Pro Leu Lys Thr Pro Tyr Phe Asp Ala Gly Ala Ser Cys Thr Glu
435 440 445

Gln Glu Met Pro Arg Tyr His Glu Leu Pro Thr Leu Glu Glu Arg Ser
450 455 460

Gly Pro Leu His Pro Gly Ala Thr Ser Leu Gly Ser Pro Ile Pro Val
465 470 475 480

Pro Pro Gly Pro Pro Ala Val Glu Asp Val Ser Leu Asp Leu Glu Asp
485 490 495

Glu Glu Gly Glu Glu Glu Glu Glu Tyr Leu Asp Lys Ile Asn Pro Ile
500 505 510

Tyr Asp Ala Leu Ser Tyr Ser Ser Pro Ser Asp Ser Tyr Gln Gly Lys
515 520 525

Gly Phe Val Met Ser Arg Ala Met Tyr Val
530 535

<210> 62
<211> 479
<212> PRT
<213> Homo sapiens

<400> 62

```
Met Ala Arg Ala Ala Ala Leu Leu Pro Ser Arg Ser Pro Pro Thr Pro
1               5                  10                  15

Leu Leu Trp Pro Leu Leu Leu Leu Leu Leu Leu Glu Thr Gly Ala Gln
            20                  25                  30

Asp Val Arg Val Gln Val Leu Pro Glu Val Arg Gly Gln Leu Gly Gly
        35                  40                  45

Thr Val Glu Leu Pro Cys His Leu Leu Pro Pro Val Pro Gly Leu Tyr
    50                  55                  60

Ile Ser Leu Val Thr Trp Gln Arg Pro Asp Ala Pro Ala Asn His Gln
65                  70                  75                  80

Asn Val Ala Ala Phe His Pro Lys Met Gly Pro Ser Phe Pro Ser Pro
                85                  90                  95

Lys Pro Gly Ser Glu Arg Leu Ser Phe Val Ser Ala Lys Gln Ser Thr
            100                 105                 110

Gly Gln Asp Thr Glu Ala Glu Leu Gln Asp Ala Thr Leu Ala Leu His
        115                 120                 125

Gly Leu Thr Val Glu Asp Glu Gly Asn Tyr Thr Cys Glu Phe Ala Thr
    130                 135                 140

Phe Pro Lys Gly Ser Val Arg Gly Met Thr Trp Leu Arg Val Ile Ala
145                 150                 155                 160

Lys Pro Lys Asn Gln Ala Glu Ala Gln Lys Val Thr Phe Ser Gln Asp
                165                 170                 175

Pro Thr Thr Val Ala Leu Cys Ile Ser Lys Glu Gly Arg Pro Pro Ala
            180                 185                 190

Arg Ile Ser Trp Leu Ser Ser Leu Asp Trp Glu Ala Lys Glu Thr Gln
        195                 200                 205

Val Ser Gly Thr Leu Ala Gly Thr Val Thr Val Thr Ser Arg Phe Thr
    210                 215                 220
```

```
Leu Val Pro Ser Gly Arg Ala Asp Gly Val Thr Val Thr Cys Lys Val
225                 230                 235                 240

Glu His Glu Ser Phe Glu Glu Pro Ala Leu Ile Pro Val Thr Leu Ser
                245                 250                 255

Val Arg Tyr Pro Pro Glu Val Ser Ile Ser Gly Tyr Asp Asp Asn Trp
            260                 265                 270

Tyr Leu Gly Arg Thr Asp Ala Thr Leu Ser Cys Asp Val Arg Ser Asn
        275                 280                 285

Pro Glu Pro Thr Gly Tyr Asp Trp Ser Thr Thr Ser Gly Thr Phe Pro
    290                 295                 300

Thr Ser Ala Val Ala Gln Gly Ser Gln Leu Val Ile His Ala Val Asp
305                 310                 315                 320

Ser Leu Phe Asn Thr Thr Phe Val Cys Thr Val Thr Asn Ala Val Gly
                325                 330                 335

Met Gly Arg Ala Glu Gln Val Ile Phe Val Arg Glu Thr Pro Arg Ala
            340                 345                 350

Ser Pro Arg Asp Val Gly Pro Leu Val Trp Gly Ala Val Gly Gly Thr
        355                 360                 365

Leu Leu Val Leu Leu Leu Leu Ala Gly Gly Ser Leu Ala Phe Ile Leu
    370                 375                 380

Leu Arg Val Arg Arg Arg Arg Lys Ser Pro Gly Gly Ala Gly Gly Gly
385                 390                 395                 400

Ala Ser Gly Asp Gly Gly Phe Tyr Asp Pro Lys Ala Gln Val Leu Gly
                405                 410                 415

Asn Gly Asp Pro Val Phe Trp Thr Pro Val Val Pro Gly Pro Met Glu
            420                 425                 430

Pro Asp Gly Lys Asp Glu Glu Glu Glu Glu Glu Glu Glu Lys Ala Glu
        435                 440                 445

Lys Gly Leu Met Leu Pro Pro Pro Pro Ala Leu Glu Asp Asp Met Glu
```

450 455 460

Ser Gln Leu Asp Gly Ser Leu Ile Ser Arg Arg Ala Val Tyr Val
465 470 475

<210> 63
<211> 412
<212> PRT
<213> Rattus norvegicus

<400> 63

Met Ala Pro Leu Ala Gly Ala Ser Arg Ser Arg Val Trp Ser Ala Gly
1 5 10 15

Leu Leu Arg Leu Leu Leu Leu Ser Cys Phe Thr Leu Gln Lys Ala Gly
20 25 30

Gly Glu Ile Ala Val Gln Val Leu Ser Asn Ser Thr Gly Phe Leu Gly
35 40 45

Gly Ser Thr Val Leu His Cys Ser Leu Ala Ser Lys Asp Asn Val Thr
50 55 60

Ile Thr Gln Leu Thr Trp Met Lys Arg Asp Pro Asp Gly Ser His Pro
65 70 75 80

Ser Val Pro Val Phe His Pro Lys Lys Gly Pro Ser Ile Ser Asp Pro
85 90 95

Glu Arg Val Lys Phe Leu Val Ala Lys Val Tyr Glu Asp Leu Arg Asn
100 105 110

Ala Ser Leu Ala Ile Ser Asn Leu Arg Val Glu Asp Glu Gly Ile Tyr
115 120 125

Glu Cys Gln Ile Ala Thr Phe Pro Thr Gly Ser Lys Ser Ala Asn Val
130 135 140

Trp Leu Lys Val Phe Ala Arg Pro Lys Asn Thr Ala Glu Ala Leu Glu
145 150 155 160

Pro Ser Pro Thr Leu Met Pro Gln Asp Val Ala Lys Cys Ile Ser Ala
165 170 175

```
Asp Gly His Pro Pro Gly Arg Ile Thr Trp Ser Ser Asn Val Asn Gly
            180                 185                 190

Ser Tyr Arg Glu Met Lys Glu Thr Gly Ser Gln Pro Gly Thr Thr Thr
        195                 200                 205

Val Ile Ser Tyr Leu Ser Met Val Pro Ser Ser Gln Ala Asp Gly Thr
    210                 215                 220

Asn Ile Thr Cys Thr Val Glu His Glu Ser Phe Gln Glu Pro Asp Gln
225                 230                 235                 240

Gln Pro Leu Ile Leu Ser Leu Pro Tyr Pro Pro Glu Val Ser Ile Ser
                245                 250                 255

Gly Tyr Glu Gly Asn Trp Tyr Ile Gly Leu Thr Asn Val Asn Leu Thr
            260                 265                 270

Cys Glu Ala Arg Ser Lys Pro Pro Pro Thr Asn Tyr Ser Trp Ser Thr
        275                 280                 285

Ala Thr Gly Pro Leu Pro Asn Ser Thr His Phe Gln Glu Asn Gly Ser
    290                 295                 300

His Leu Leu Ile Ser Thr Val Asp Asp Leu Asn Asn Thr Ile Phe Val
305                 310                 315                 320

Cys Lys Ala Ile Asn Ala Leu Gly Ser Gly Gln Gly Gln Val Thr Ile
                325                 330                 335

Leu Val Lys Glu Ala Ser Glu Ile Leu Pro Pro Lys Thr Ser Leu Gly
            340                 345                 350

Thr Gly Tyr Ile Ile Ala Ile Val Phe Cys Val Leu Ile Ile Gly Val
        355                 360                 365

Val Ala Gly Ile Val Phe Trp Lys Tyr Arg Arg Gly Cys Gly Arg Gln
    370                 375                 380

Ser Arg Thr Leu Asp Arg Glu Asn Val Arg Tyr Ser Ala Ala Asn Gly
385                 390                 395                 400
```

Val Ser Val Pro Asn Val Glu Thr Asn Asn Leu Arg
405 410